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THE VIABILITY OF WEED SEEDS AT VARIOUS STAGES OF MATURITY

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(With 4 Text-figures)

INTRODUCTION

THE practice of cutting down plants before seed is produced is recommended for the eradication of certain weeds, with the precaution that cutting should be done early in order to prevent the maturation of seed on the cut shoots lying on the ground. Certain plants appear to mature their seeds after being cut, but whether these seeds are capable of germination is uncertain in many cases.

More detailed information on the stage of growth at which weeds are capable of producing viable seeds on the cut shoots is desirable, not only in connexion with the cutting of large weeds during weeding operations but, also, in connexion with the haying of grass containing weed species, and the cultivation of arable land. Fleischmann (1928) has shown that viable seed can be obtained from wheat if this is harvested after about one-third of the period between fertilization and the time at which maturity would normally occur. The object of the present investigation was to determine the time after which weeds, when cut, would similarly produce viable seeds which might find their way to the soil and germinate.

METHODS

Common weeds were cut down near the base of the plant and allowed to dry in the sun. Samples were obtained at stages of development of each species varying from that at which the flower was still in the bud, through the open-flower stage, to various stages of maturity of the seed after fertilization. Samples of "dead-ripe" seed matured on the growing plant were obtained from each species for comparison. All samples were taken from at least ten distinct plants of each species, except in the case of thorn apple (*Datura Stramonium* L.), of which only seven plants were obtained. The seeds of each stage of maturity so obtained were mixed before testing. Germination tests were made using 100 seeds on

damp filter papers in Petri dishes, each test being performed in duplicate. The tests were usually made in an incubator at a temperature of 20–22° C. The fact that some seeds germinate better in the light and with fluctuating temperature (Warington, 1936) was taken into account, and, in some cases where germination was slow in the incubator, more rapid germination was obtained by standing the dishes on a laboratory bench near a window under conditions of fluctuating temperature and illumination.

With some species germination did not reach a high percentage until after a period of storage; therefore the seeds of these plants were stored in glass bottles in an open garden shelter and tested periodically until germination occurred freely or, in the case of the immature seeds, until it became apparent that germination would not occur.

As the object of the investigation was to demonstrate the viability of seeds harvested at various stages of development, and not to determine the maximum germination possible for the samples, tests were in some cases discontinued after a germination capacity of 50% or more had been demonstrated, although it is possible that, if such samples had been tested at later dates, higher figures would have been obtained.

Observations

(i) *Plants belonging to the family Compositae*

Many of the more common weeds belonging to the family Compositae were obtained at well-defined stages of growth:

(*a*) When all the flowers in the capitulum were still in bud.

(*b*) When the bifid stigmas were open.

(*c*) When the corolla was dying and the pappus beginning to extend.

(*d*) When dead-ripe fruits had formed.

It has been shown by La Rue (1935) that dandelions (*Taraxacum vulgare* Schrank.) may be cut at all stages of growth until the pappus begins to extend and, if the cut plants are left drying on a lawn, they will not produce seeds. Our experiments with this species gave similar results, but, within the family as a whole, results varied considerably.

The results of the germination tests of the species examined are summarized in Table I. For each species the figures were obtained by a test made within 2 weeks after collection of the seeds.

Ragwort. Providing the flowers were open and the stigmas visible viable seed was produced. When cut in the bud condition many of the buds on drying produced a white fluffy mass of pappus, but the "seed" attached was not capable of germination.

Table I

Germinability of seeds of Compositae

	Percentage germination		
	Dead ripe	Cut in flower	Cut in bud
Ragwort (*Senecio Jacobaea* L.)	72	80	0
Sow thistle (*Sonchus oleraceus* L.)	100	100	0
Groundsel (*Senecio vulgaris* L.)	90	35	0
Sea aster (*Aster Tripolium* L.)	90	86	0
Dandelion (*Taraxacum vulgare* Schrank.)	91	0	0
Cat's ear (*Hypochaeris radicata* L.)	90	0	0
Creeping thistle (*Cirsium arvense* Scop.)	38	0	0

Common sow thistle. All open flowers appear to be capable of producing viable seeds on drying.

Groundsel. A small but, from a weed infestation point of view, a very appreciable proportion of the seeds produced by the open cut flowers are capable of germination. The percentage of the seeds germinating in this sample did not increase during storage.

Sea aster. Although not of importance as a weed this species was included for comparison with other pappus-bearing species. The flowers ripened their seeds after the plant had been cut down.

Dandelion. Whilst ripe seeds gave a high percentage germination, flowers, when cut down, unlike *Senecio* did not produce any viable seeds, and the pappus did not elongate.

Cat's ear. A common weed in hay in some districts, gave results similar to dandelion.

Creeping thistle. The germination capacity, even of ripe seeds, was low and remained so even after storage. Very few fruits were produced by plants cut down when the flowers were in bloom, and none of these was capable of germination. Although, in external appearance, they were like the normally ripened fruits, they consisted almost entirely of an empty pericarp without any fully developed embryo.

A preliminary examination of spear thistle (*Cirsium lanceolatum* Scop.) showed that the plants cut in the flowering condition produced few fruits and that these, although looking like normal fruits, did not contain a mature embryo and would not germinate.

(ii) *Plants belonging to the family Gramineae*

Two grass species common in meadows and, therefore, frequently present in hay were examined, namely meadow barley grass (*Hordeum nodosum* L.) and soft brome grass (*Bromus mollis* L.). In each case plants cut in the flowering condition (i.e. when stigmas were visible)

did not ripen their grains, but plants cut after the stigmas had withered when the grains were in the milk-ripe condition (in each case the plants were still green at this stage) produced seeds with a high percentage germination. The percentage figures obtained within 2 weeks of harvesting the plants were:

	Dead ripe	Milk ripe
Meadow barley grass (*Hordeum nodosum* L.)	94	90
Soft brome grass (*Bromus mollis* L.)	96	81

(iii) *Other species*

Curled dock (Rumex crispus *L.*).

The plants of this species were cut down in September, but it was not until 3 months later that any of the seeds obtained would germinate in the incubator, although it has since been found that the seeds will germinate within a month under conditions of fluctuating temperature and illumination. Plants cut in the flowering condition did not ripen seed, but plants in which the seed had begun to form and was in the milk-ripe condition ripened viable seeds. On such plants the perianth around the fruits was still green and the leaves had not begun to die.

The percentage figures obtained for germination in the incubator after a period of dormancy lasting for 3 months were:

Dead-ripe seeds	...	84
Milk-ripe seeds	...	88

Thus, it is advisable to cut this plant very early before the seeds have begun to form.

Preliminary tests on broad dock (*Rumex obtusifolius* L.) indicate that this species behaves in a similar manner.

Thorn apple (Datura stramonium *L.*).

Although only a casual weed this plant has yielded interesting results and was, therefore, thought worthy of inclusion. The results of the tests are shown in Fig. 1. Dead-ripe seeds, immediately after collection from the dried plant, gave 100% germination. From the partly ripe green capsules present on the plant, 35% of the seeds collected were green and wrinkled and incapable of germination; the remainder were black like the normally ripened seeds, and their germination capacity immediately after drying was 67%. A month later the germination capacity of the dead-ripe seeds was nil, whilst the germination capacity of the black seeds from the immature capsules had reached 100%. The seeds were tested at intervals throughout the winter and the following summer, and the germination capacity of the "immature seeds" remained at 100%,

whilst that of the mature seeds remained nil. It was found, however, that when the seed coat of the mature seeds was first cracked 100% germination could be obtained. Thus, it seems that if dead-ripe seeds are placed under conditions suitable for germination immediately after being shed they germinate freely but, after a few weeks, the seed coat of ungerminated seeds becomes impermeable and the seeds assume a

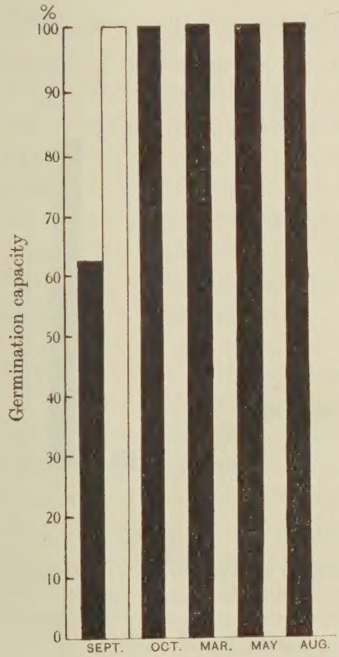

Fig. 1.

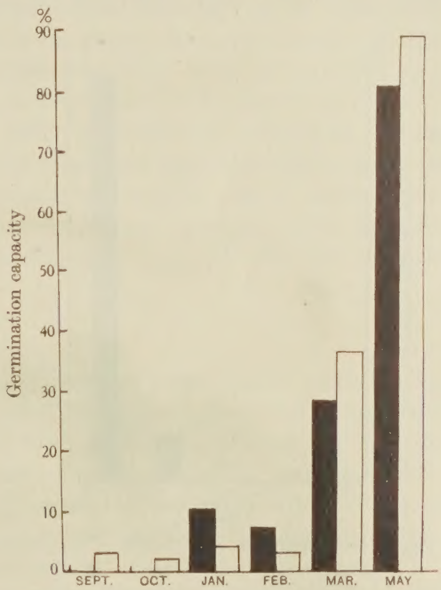

Fig. 2.

Fig. 1. Germination of stored seeds of thorn apple (*Datura stramonium* L.) collected in September. ☐ dead-ripe seeds; ■ seeds from immature green fruits.

Fig. 2. Germination of stored seeds of shepherd's purse (*Capsella Bursa pastoris* Med.) collected in September. ☐ dead-ripe seeds; ■ seeds from sun-dried large green fruits.

dormant condition. The seed coat of the "immature seeds", on the other hand, does not become impermeable and germination continues freely.

Shepherd's purse (Capsella Bursa pastoris *Med.*).

The seed capsules were divided into four groups: dead ripe, large green, medium green, and small green. The last two groups did not produce any viable seeds, but the first two both produced seeds capable

of germination after remaining dormant through the winter. The results are shown in Fig. 2. There was little germination of either the ripe seed or the immature seed until March and in May the germination of each was high and vigorous.

Corn speedwell (Veronica agrestis *L.*).

As in the case of the last species only the largest of the immature fruits produced viable seeds, and the germination of these was closely

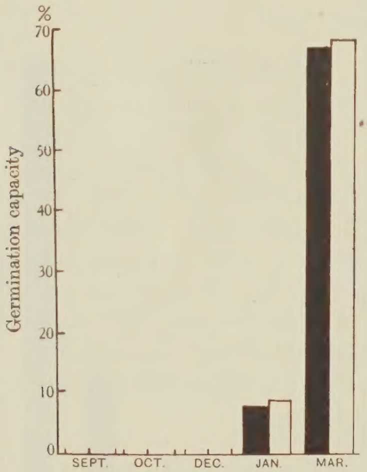

Fig. 3.

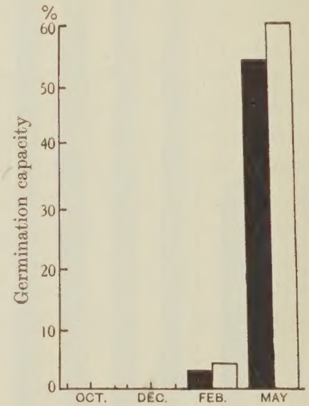

Fig. 4.

Fig. 3. Germination of stored seeds of corn speedwell (*Veronica agrestis* L.) collected in September. □ dead-ripe seeds; ■ seeds from sun-dried large green fruits.

Fig. 4. Germination of stored seeds of common chickweed (*Stellaria media* Vill.) collected in September. □ dead-ripe seeds; ■ seeds from sun-dried green fruits.

comparable with that of the dead-ripe seeds. In each case seeds collected in September did not give vigorous germination until March (Fig. 3).

Common chickweed (Stellaria media *Vill.*).

The flowers did not produce seeds after being cut down, but any green immature fruits present at the time of cutting ripened seed capable of germination, like the dead-ripe seeds, after a period of dormancy through the winter (Fig. 4).

Poppy (Papaver dubium *L.*).

Dead-ripe seeds were tested regularly for a year after collection but did not germinate, whilst seeds from green sun-dried capsules collected

in September, after remaining dormant in the winter, gave an appreciably large germination in February and throughout the summer.

The percentage figures obtained were:

	Sept.	Oct.	Dec.	Feb.	May	Aug.
Dead-ripe seeds	0	0	0	0	0	0
Seeds from immature green capsules	0	0	0	46	36	44

Thus, it would appear that seed from immature green capsules is capable of producing new plants in the season after its production, whilst dead-ripe seed passes into a dormant condition. It should be added that some of the dead-ripe seed was sown in soil in pots placed outside, and this failed to germinate in the first year. Exposure of samples, during germination tests, to fluctuating temperature and illumination failed to encourage germination in the dead-ripe samples, or to increase the germination capacity of the "immature green" samples. As in the case of *Datura*, when the seed coat of the dead-ripe seeds was first cracked, germination occurred freely.

Common nettle (Urtica dioica *L.*).

Samples cut in the flowering condition when the stigmas were showing did not ripen seed, but plants cut when the seeds were in the milk-ripe condition, and the perianth still fresh and green, ripened seed of high germination capacity. These seeds, together with the dead-ripe seeds, showed low germination capacity during the first month after collection, but, a month later, germination in both cases was vigorous. The percentage figures obtained were:

	Sept.	Nov.
Dead-ripe seeds	27	77
Milk-ripe seeds	22	70

DISCUSSION

It is impossible to make any natural grouping of plant species with regard to the ripening or non-ripening of their seeds when the plants are cut in an immature condition. Within the family Compositae alone some species ripen seeds when cut down in the flowering condition; others produce apparently normal fruits which, however, contain no germinable seed; whilst others merely shrivel, making no progress toward the production of seeds. In the species examined in other families it seems to be necessary for the seeds to be present in the milk-ripe state before the plant is cut down if viable seeds are to be produced.

In the majority of species examined the germination of seeds harvested in the early stages of development occurs at about the same time as that of dead-ripe seeds and, in species in which the dead-ripe seeds pass through a period of after-ripening before germination is possible, the "immature" seeds undergo a similar period of after-ripening. *Papaver dubium* behaved in abnormal manner in this respect, the "immature" seeds beginning to germinate after 4 months' storage, whilst untreated dead-ripe seeds failed to germinate during the 12 months of the experiment. The fact that the dead-ripe seeds germinate if the seed coat is split indicates that dormancy is due only to the condition of the seed coat, and it is probable that further tests would show that the physiological after-ripening of the embryo of dead-ripe seeds takes about the same length of time as that of the "immature" seeds, and that continued dormancy is enforced by the condition of the seed coat. Takiguti (1930) has demonstrated a similar effect in rape seed (*Brassica napella*), in which seed harvested in the milk-ripe state germinated freely, whilst seed obtained after the brown pigment had developed in the seed coat germinated in the incubator only if the seed coat were first removed. The ripe seed in this case could, however, be induced to germinate by submitting it to fluctuating temperature conditions during germination. Fluctuating temperature and illumination appeared to have no effect on dead-ripe *Papaver* seeds, but it is possible that variations of temperature other than those occurring outside, in the case of the seeds sown in plant pots, or in the laboratory in the case of the Petri dish tests, may bring about germination of the dead-ripe seeds within 12 months. *Datura* is similar to *Papaver* in behaviour, save that the embryo is capable of growth soon after harvesting and, in the case of the dead-ripe seeds, the changes in the seed coat which induce dormancy occur a short time after the seeds are shed.

The effect of fluctuating temperature and illumination on the germination of the various species has not been examined in detail, since the object of the investigation was to ascertain the viability of "immature" seeds. Only in cases where germination was very low in the incubator have variable conditions of temperature and illumination been tested. Gardner (1921) has already shown that light encourages the germination of *Rumex crispus*. Warington (1936) has shown a beneficial effect of fluctuating temperature combined with illumination on several weed species, and suggests that fluctuating temperature is probably the more important; this would appear to be supported by Gassner's experiments (1930) which show that many seeds which are aided to germinate

by light also germinate readily if submitted to intermittent temperature in darkness. Fluctuating temperature and light together were found to have a beneficial effect on both *Rumex* species; these, however, also germinated freely in the incubator after a short period of storage. Other species tested and found difficult to germinate in the incubator have not responded to fluctuating temperature and illumination. Some species, such as hemp nettle (*Galeopsis speciosa* Mill.), knotgrass (*Polygonum aviculare* L.), and corn marigold (*Chrysanthemum segetum* L.), have been tested periodically for a period of 12 months, and neither "immature" nor dead-ripe seeds have germinated. These species may undergo a prolonged after-ripening, or the conditions under which the tests were made may have been unsuitable for the germination of their seeds.

Brenchley & Warington (1930) found an apparent periodicity in the germination of seeds of various weed species present in field soil and consisting of seeds of unknown age. The tests performed on stored seeds have not been continued for a sufficiently long period to show whether such periodicity exists in seeds of any one harvest year under these storage conditions.

Summary

1. Certain plants within the family Compositae when cut down in the flowering condition produce viable seeds; others do not. The first group includes: *Sonchus oleraceus*, *Senecio Jacobaea*, *S. vulgaris* and *Aster Tripolium*, whilst the second group includes: *Taraxacum vulgare*, *Hypochaeris radicata*, *Cirsium arvense* and *C. lanceolatum*.

2. Certain species in other families produce viable seeds when cut down at various stages of maturation of the fruits following fertilization.

3. When the mature seeds exhibit a prolonged dormant period, the seeds harvested in an immature state exhibit a similar period of dormancy.

4. In *Papaver dubium* and *Datura stramonium*, the immature seed germinated more readily than the fully ripened seed, owing to the impermeability of the seed coat of the latter.

5. A combination of fluctuating temperature and illumination improved the germination of the two *Rumex* species when tested soon after harvesting, but had no effect on other species found difficult to germinate in an incubator at constant temperature. Three months after harvesting of the *Rumex* species the seeds germinated equally well in the incubator at constant temperature.

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THE DEVELOPMENT OF SAINFOIN IN ITS SEEDING YEAR

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(With Plates XVIII and XIX and 7 Graphs)

CONTENTS

Introduction

Cultivated sainfoin is an agricultural crop of some importance in England, particularly on the chalky soils of the south and east where it is indigenous. Unfortunately, the acreage grown is unknown, as in the Ministry of Agriculture's statistics it is included in "rotation grasses and clovers". It is probably true that it is grown less at the present time than formerly, but it is still highly valued for pasture, hay and forage. There is some confusion as to its nomenclature, but it can best be defined as *Onobrychis sativa* Lam. (*sensu stricto*). Two varieties are in common use, common or single-cut sainfoin, *O. sativa* var. *communis* Ahlefeld, and giant or double-cut sainfoin, *O. sativa* var. *bifera* Hort. Common sainfoin is a long-lived plant which reaches its maximum yield in its third year. Leys of 20 years have been recorded, but usually 7 or 8 years is considered satisfactory, the end of a ley being determined by the invasion of weeds. Common sainfoin produces flowering stems in May or June and the ley can then be cut for hay. In its subsequent growth rosettes of leaves are formed but no stems, hence the aftermath can be used for grazing only. Giant sainfoin is a shorter lived plant and is used for short leys of 1 or 2

years. After cutting, it again sends up flowering shoots, giving a second cut of hay and, sometimes, it may flower a third time and give a third hay crop. A third variety has been described under the name of three-cut sainfoin characterized by thrice flowering, but the distinction is not constant and this form should be included in the giant variety (Zade, 1933).

In order to avoid excessive repetition, common sainfoin (*O. sativa* var. *communis*) will be referred to throughout this paper as "common", and giant sainfoin (*O. sativa* var. *bifera*) as "giant".

In spite of its agricultural importance, sainfoin has received little scientific attention as compared with other forage crops such as lucerne and clover. Little is known, for example, of the differences between the two varieties, giant and common, or of the development of sainfoin from seedling to mature plant. Our present knowledge of its seeding year behaviour may fairly be summarized as follows:

After the kidney shaped cotyledons reach the soil surface, foliage leaves with various numbers of leaflets are produced. The first foliage leaf is simple, the second and third are trifoliate, and the later leaves are pinnate with from six to twelve pairs of leaflets and a terminal one. Short lateral branches are formed and the plant forms a rosette which tends to be more prostrate in common than in giant. Later, branches may elongate and bear inflorescences (Percival, 1936; Robinson, 1937; Fleischmann, 1932; Rees, 1928, 1931, 1932). According to Rees, the amount of flowering in the seeding year is variable and depends partly on the variety and partly on the time of sowing. The present work was undertaken with a view to filling in some of the details of this picture by a close study of the growth of sainfoin plants in their seeding year, with particular reference to differences between the giant and common varieties.

Experimental methods

The work was carried out at the University of Reading's Agricultural Botanic Garden at Shinfield, Berks. Samples of giant and common sainfoin were sown in rows in the open in 1935 and 1937 and kept under observation throughout each season. In 1935 the sowing date was 29 March, but in 1937, owing to the failure of an earlier sowing, the date was 18 May. A small quantity of seed was also sown in an unheated glasshouse in February 1937. Altogether, thirteen lots of giant and thirteen lots of common were sown. Counts were made of the number of leaflets in successive foliage leaves of the seedlings up to the appear-

ance of the sixth leaf. Beyond this it was impossible to determine the order of the leaves owing to the development of lateral branches. The figures for leaflet numbers were obtained by combining the results from all the lots of seed sown in 1935 and 1937. For convenience, the number of leaflets in successive leaves is indicated by a group of numbers, e.g. if a seedling at the four leaved stage has the combination 1.3.3.5, this means that the first leaf is simple, the second and third leaves have three leaflets each, and the fourth has five leaflets. In 1937, the number of tillers in 400 spaced seedlings was counted.

GERMINATION

In the case of milled seeds, i.e. true seeds, germination is of the epigeal type normally found in the Leguminosae. The radicle breaks through the testa and elongates; the hypocotyl then elongates, withdrawing the cotyledons from the testa and carrying them to the soil surface where they open out and lie flat on the surface. The cotyledons are thick and fleshy, kidney shaped, with a flat upper surface and a convex lower surface, and turn dark green on exposure to light.

In the case of unmilled seed, i.e. seeds inside the indehiscent pericarp, the first sign of germination is the protrusion of the radicle through the pericarp as figured by Nobbe (1876). The radicle always emerges at the same point. On the surface of the fruit is a network of raised vascular ridges, and the radicle forces its way through the large mesh near the upper end of the ventral suture (Pl. XVIII, fig. 1). The swelling of the seed then causes the pericarp to split along the dorsal suture, and the hypocotyl elongates and carries the cotyledons out of the pericarp through this split (Pl. XVIII, fig. 1), leaving the testa within the pericarp. The ventral suture was never observed to split. In consequence of this method of germination, the radicle is encircled by the mesh of the tough vascular ridges and it may be some weeks before this is broken, with the result that a constriction is produced in the young root. When a seedling is pulled up at a later stage the pericarp can still be found firmly attached (Pl. XVIII, figs. 2, 3).

While the majority of unmilled seeds germinate in this way, a few are unable to break through the pericarp, but this does not necessarily prevent germination. In such cases, the swelling of the seed splits the dorsal suture and the radicle can grow out through the split. The seedling is thus able to free itself completely from the pericarp.

In 1935 the cotyledons had appeared above ground by 23 April, 26 days after sowing. This is rather a long time, but conditions of tilth

and weather were unfavourable. Rees (1931) found that when seed was sown at the end of March, the seedlings appeared within a week.

The phenomenon of delayed germination is well known in grasses and clovers and, in view of its importance in the case of seeds sown for long leys, it is interesting to know whether or not common sainfoin exhibits this trait. Delayed germination in sainfoin was observed 200 years ago by Jethro Tull (1733), who found that if sainfoin was sown with a nurse crop many seeds did not germinate till the following spring. Rees (1931) found indications of it in his experiments and, in the present work, definite evidence was found. Seed was sown in May 1937 and fresh seedlings continued to appear until October, and again from February onwards the following year, more abundantly in common than in giant. Unfortunately, beyond this statement of fact no data were obtained.

The first six foliage leaves

The first six foliage leaves all arose at the same level, between the cotyledons, the epicotyl remaining short. The dates of their appearance in 1935 were as follows:

	Date	No. of days after appearance of cotyledons
Cotyledons	23 April	0
1st leaf	4 May	12
2nd leaf	12 May	20
3rd leaf	25 May	33
4th leaf	1 June	40
5th leaf	11 June	50
6th leaf	25 June	64

In 1937 the date of sowing was later and growth was more rapid.

The first leaf is usually simple, but a number of seedlings were found with two, three or four leaflets. In common there was a slightly higher proportion of simple leaves than in giant (Table I, Graph 1). Giant had an average of 1·21 and common of 1·16 leaflets per leaf, but the difference is not significant. The first foliage leaf has a long erect petiole arising from between the cotyledons. In a simple leaf the blade is broadly ovate and in two- and three-foliate leaves the lateral leaflets are narrower. Seedlings with simple and two-foliate leaves are shown in Pl. XVIII, fig. 2.

In the majority of seedlings the second leaf was trifoliate, but leaves with one, two, four or five leaflets were also found (Pl. XVIII, fig. 3). There was little difference between the two varieties (Table I, Graph 2). Both had a preponderance of trifoliate leaves, but the proportion of five-foliate leaves was slightly higher and of simple leaves slightly lower

in giant. The mean number of leaflets per leaf in giant, 3·04, was slightly but significantly greater than in common, 2·95.

Table I

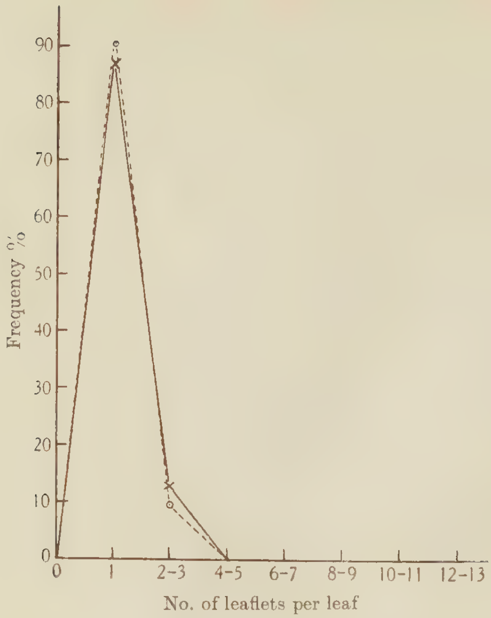
Percentage frequency of leaflet numbers in the first six foliage leaves of sainfoin seedlings

	% frequency											
	1st leaf		2nd leaf		3rd leaf		4th leaf		5th leaf		6th leaf	
No. of leaflets	Giant	Common	Giant	Common	Giant	Common	Giant	Common	Giant	Common	Giant	Common
1	87·1	90·3	0·7	1·8	0·1	0·4						
2	4·4	3·2	3·1	4·6	0·8	1·1	0·2	0·1	—	—	—	—
3	8·4	6·5	91·0	91·6	57·2	72·7	19·2	41·5	3·2	13·6	0·2	1·5
4	—	Occ.	1·4	0·8	6·8	5·2	4·9	6·1	1·3	2·7	0·2	0·6
5	—	—	3·8	1·3	32·6	20·2	54·0	44·0	41·4	54·5	13·7	36·1
6	—	—	—	—	9·8	Occ.	3·1	1·2	4·4	2·7	1·2	3·4
7	—	—	—	—	1·6	9·4	14·9	6·1	29·2	19·7	35·8	35·5
8	—	—	—	—	—	—	0·5	0·1	2·4	0·8	3·7	1·9
9	—	—	—	—	—	—	3·0	0·9	12·6	4·8	28·4	14·5
10	—	—	—	—	—	—	0·1	—	0·6	0·4	1·9	0·7
11	—	—	—	—	—	—	0·1	—	4·3	0·8	14·4	4·0
12	—	—	—	—	—	—	—	—	—	—	Occ.	0·1
13	—	—	—	—	—	—	—	—	—	—	0·5	1·4
14	—	—	—	—	—	—	—	—	—	—	—	—
15	—	—	—	—	—	—	—	—	—	—	Occ.	0·2
Mean	1·21	1·16	3·04	2·95	3·80	3·46	4·48	4·28	6·40	5·40	7·99	6·75
S.E.	0·016	0·014	0·011	0·010	0·023	0·020	0·036	0·030	0·044	0·038	0·052	0·051
No. of leaves counted	1326	1459	1756	1858	1910	1923	1840	1803	1696	1652	1327	1352

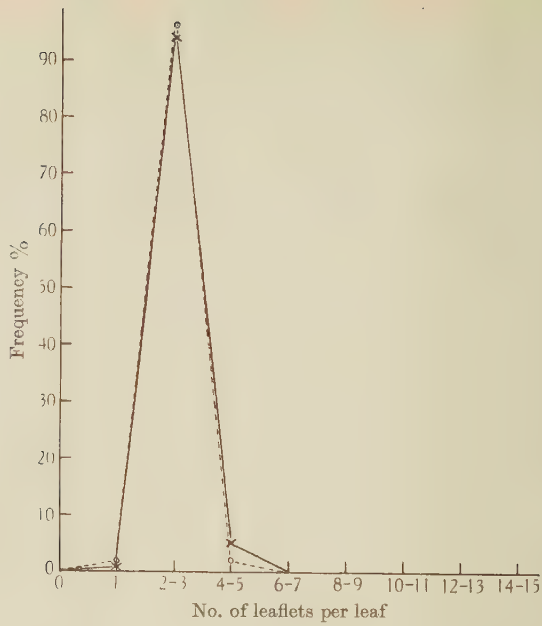
Third leaves with three and five leaflets were most frequent, but there were also leaves with one, two, four, six and seven leaflets (Pl. XVIII, fig. 4, Table I and Graph 3). A definite difference between giant and common seedlings had at this stage become apparent, the former tending to have a greater number of leaflets. Giant had 57·2 % of its leaves trifoliate and 32·6 % five-foliate, while the corresponding percentages for common were 72·7 and 20·2. The mean number of leaflets per leaf in giant was 3·8 and in common 3·46, the difference being significant.

In the fourth leaf, three and five leaflets were again most usual, but leaves with leaflets varying from two to eleven were found (Pl. XVIII, fig. 5, Table I and Graph 4). The difference between giant and common was maintained. While giant had a higher proportion of leaves with five or more leaflets, common had a higher proportion with less than five. The mean number of leaflets per leaf was again significantly different, giant having 4·48 and common 4·28.

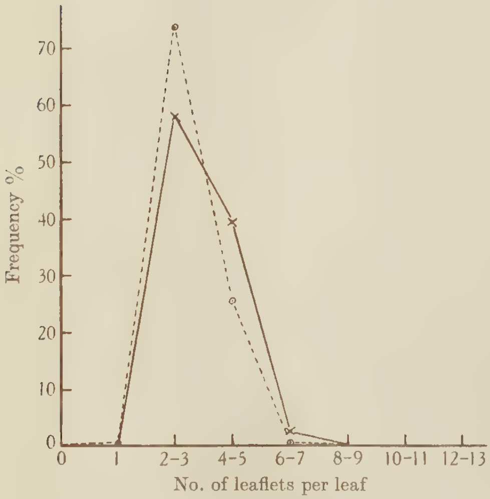
The number of leaflets in the fifth leaf varied from three to eleven, but the most frequent numbers were five and seven (Pl. XVIII, fig. 6,



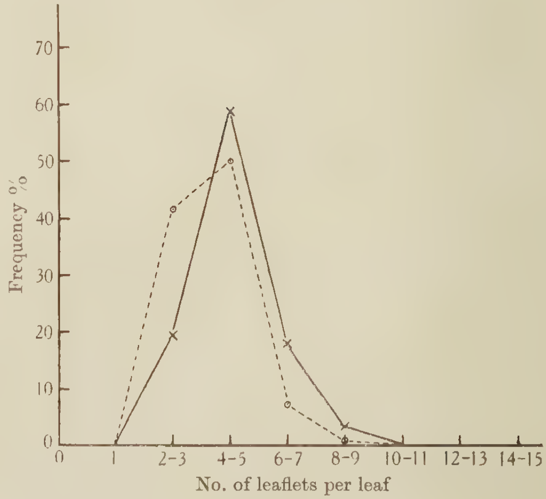
Graph 1. First leaf.



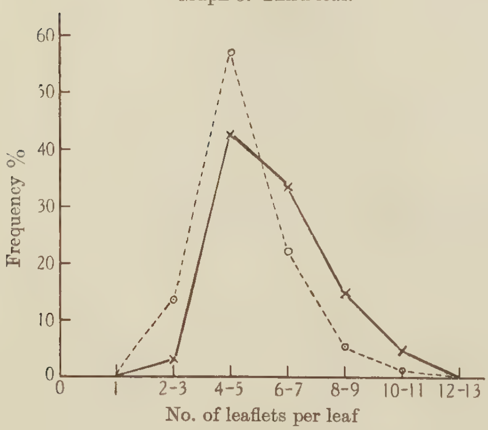
Graph 2. Second leaf.



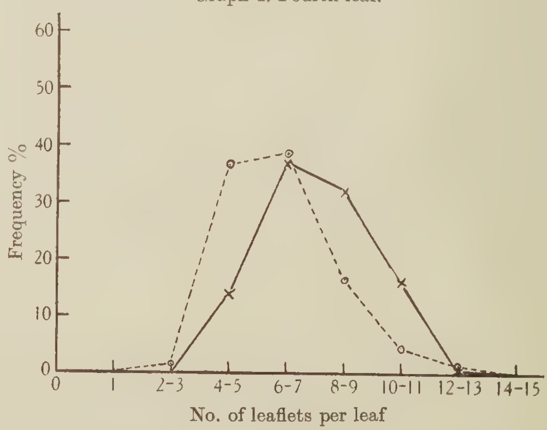
Graph 3. Third leaf.



Graph 4. Fourth leaf.



Graph 5. Fifth leaf.



Graph 6. Sixth leaf.

Table I and Graph 5). The higher numbers were more frequent in giant than in common. Giant had an average of 6·4 leaflets per leaf and common 5·4, the difference again being significant.

The number of leaflets in the sixth leaf was found to vary from three to fifteen, the most usual numbers being five, seven and nine (Pl. XIX, fig. 7, Table I and Graph 6). In both giant and common the most frequent number was seven, but common had a higher proportion of leaves with less than seven leaflets than giant. The mean numbers of leaflets per leaf were 7·99 for giant and 6·75 for common; the difference is significant.

From these figures it is evident that, as between individual plants, there is very considerable variation in the number of leaflets in each leaf. For example, the number of leaflets in the sixth leaf varied from three to fifteen. The most frequent combinations were 1.3.3.5.5.5 and 1.3.3.5.5.7. Other frequent combinations were 1.3.3.3.5.5, 1.3.3.3.5.7, 1.3.5.5.7.7 and 1.3.3.5.7.9. These, however, are only a few of the innumerable combinations actually present.

With the exception of the first leaf, giant had a significantly higher number of leaflets per leaf than common. It is doubtful, however, if much value can be placed on the actual mean figures obtained, even though the differences were significant statistically, as the variation between different samples examined was so great. Table II gives the average number of leaflets per leaf for the twenty-two samples in which counts were made. As an example, the average number of leaflets in the sixth leaf varied from 6·7 to 9·7 in giant and from 5·9 to 8·7 in common for different lots and, taking individual plants, the number varied from three to fifteen. The statement that the average numbers of leaflets in the sixth leaf of giant and common are 7·99 and 6·75 respectively is, in these circumstances, meaningless, and the only conclusion that may be drawn is that there is a tendency for giant seedlings to have a greater number of leaflets in each leaf than common seedlings.

It will be noticed that leaves with even numbers of leaflets appeared consistently. A normal leaf has an equal number of leaflets on either side, more or less paired, and a terminal leaflet. In leaves with an even number of leaflets, either one of the laterals or the terminal leaflet is missing.

Graphs 1–6. Percentage frequency distribution of leaflet numbers in successive foliage leaves. —— giant; - - - - - common.

Table II

Mean number of leaflets in the first six foliage leaves of different lots of sainfoin seedlings

Sample	Mean number of leaflets					
	1st leaf	2nd leaf	3rd leaf	4th leaf	5th leaf	6th leaf
Giant A	1·4	3·0	4·1	5·8	7·7	9·7
B	1·6	3·0	4·1	5·8	7·6	9·6
C	1·6	3·1	4·3	5·5	7·0	9·0
E	1·7	3·2	4·5	5·9	7·6	9·3
F	1·9	3·3	4·8	6·2	7·9	9·5
G	2·1	3·2	4·5	6·1	7·9	9·7
J	1·1	3·0	3·4	4·3	5·4	7·2
K	1·2	3·0	3·4	4·2	5·2	6·7
L	1·4	3·0	3·3	4·7	6·0	8·0
M	1·3	3·1	3·9	5·4	6·5	8·5
N	1·7	2·5	3·3	4·7	6·0	7·5
Common A	1·4	2·8	3·9	5·3	6·9	8·5
B	1·5	3·1	3·7	4·6	5·7	6·7
C	1·4	3·0	3·6	4·6	5·7	6·7
F	1·5	3·0	3·9	4·9	6·0	7·3
G	1·4	3·0	4·0	5·2	6·5	8·1
H	1·5	3·2	4·5	5·7	7·2	8·7
J	1·1	2·9	3·1	3·7	4·7	6·0
K	1·1	2·9	3·7	4·4	5·3	6·5
L	1·4	2·9	3·2	4·7	6·1	7·8
M	1·0	2·9	3·2	3·8	4·7	5·9
N	1·3	3·1	2·8	4·4	4·9	7·0

Subsequent growth

No further counts of leaflets were made after the sixth leaf. So far, there was little obvious difference between the two varieties, giant and common. In both varieties the internodes remained short and the leaves arose at the same level forming a rosette. Superficially, the plots of giant appeared more luxuriant, and the counts have shown that this was due to the greater number of leaflets per leaf in that variety. From this stage onwards, however, there was considerable divergence in the development of the two varieties.

(*a*) *Common sainfoin.* In common sainfoin the internodes remained short throughout the season and new leaves continued to be formed on the short main stem. Soon after the formation of the sixth leaf, lateral buds began to develop. These laterals produced numerous leaves but their internodes normally remained short, thus giving a more dense appearance and accentuating the rosette habit. An occasional tiller was found in which the internodes had elongated, producing a prostrate lateral branch up to 6 in. in length, but this was exceptional. The plants remained in this condition until the end of the season and never flowered.

Rees (1932) found that some lots of sainfoin, which purported to be of the common variety, flowered in the seeding year, particularly continental strains. His work suggests, however, that these were mixtures of giant and common types and that the true common does not flower in the seeding year. Of the thirteen lots of common, including one of French origin, sown at Shinfield none showed any flowering.

In the autumn two types of plant could be distinguished, prostrate and semi-erect. In the prostrate type the leaves of the main stem and of the tillers all lay close to the ground as shown in Pl. XIX, fig. 8. In the semi-erect type the leaves of the main stem and of the tillers were produced at all angles from horizontal to erect as shown in Pl. XIX, fig. 9. The two types are not absolutely distinct, but merge into each other. Nevertheless, a population of plants can be separated into the two types quite readily. The angle of the leaves of the tillers is determined by the angle at which the tillers leave the main stem. Counts made in September 1937 showed that 46·5% of the common plants were of the prostrate type, and 53·5% of the semi-erect type.

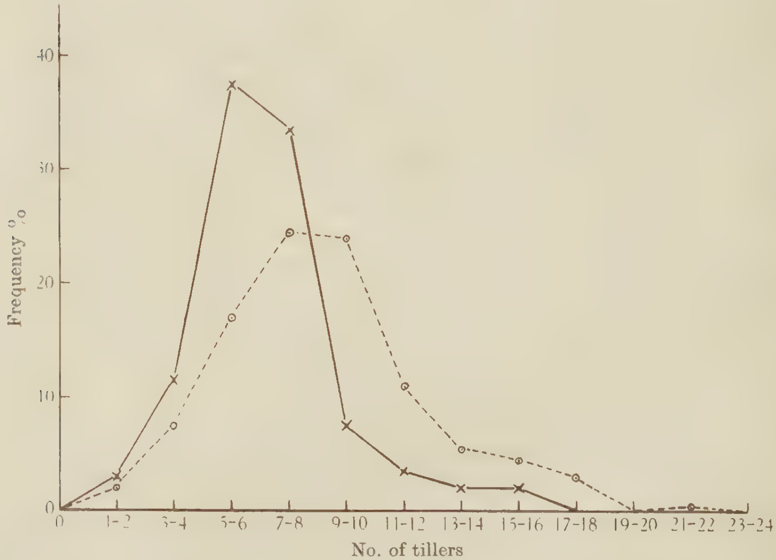
(*b*) *Giant sainfoin.* In giant sainfoin the subsequent behaviour was variable. In some plants the internodes remained short as in common, in others the internodes elongated and the plants assumed an erect habit. Most of these erect plants flowered. The rosette plants were similar to the semi-erect type of common (Pl. XIX, fig. 10). They behaved in the same way and did not flower.

The internodes of the erect giant plants began to elongate after the production of the sixth leaf. Elongation took place in the upper internodes, the first four to six internodes remaining short. Pl. XIX, fig. 7*a* shows an erect giant seedling with eight leaves, the first four arising at the same level. Lateral buds developed in the axils of the lower leaves and these tillers either elongated, or remained short and produced a tuft of leaves at the base of the plant. Pl. XIX, fig. 11 shows an erect plant with two lateral branches, one of which has elongated and the other has remained short. The elongated laterals frequently grow out horizontally for about an inch and then turn abruptly upwards. In the axils of the upper leaves either inflorescences or branches may be produced. The height of an erect plant is about 15 in.

The plots were cut on 21 August in 1935 and on 31 July in 1937. After cutting, lateral buds developed rapidly. In the case of the rosette plants, they retained this habit after cutting. In the case of the erect plants, the tillers either remained short producing a rosette habit, or else elongated giving an erect habit again.

Flowering in the seeding year is very variable. In 1935 flowering began on 1 July and only 3·3% of the plants flowered. After cutting, all the plants assumed the rosette habit and none flowered. In 1937 the flowering date was 20 July and 93·5% of the plants flowered. After cutting, practically all the plants produced erect stems and 94·5% flowered a second time on 30 August.

This variability is reflected in the little information available as to the proportion of giant plants flowering in their seeding year. Fleischmann (1932) records that, in plots, 6% of the plants flowered but,



Graph 7. Percentage frequency distribution of tiller numbers.—— giant; - - - - - common.

according to Klapp (1932), most of the sainfoin grown in Germany shows flowering in up to 100% of plants. According to Rees (1928), giant usually flowers abundantly once or twice in its seeding year in South Wales, but flowering can be influenced by the time of sowing. If sowing takes place before the end of May the seedlings will flower but, if sowing is delayed till after May, flowering will be reduced or prevented. It is probable that flowering in the seeding year is influenced to a large extent by weather conditions. Sainfoin cannot be grown successfully in this country north of the Humber; therefore, even in the south of England, the conditions must be very close to the limits which sainfoin

will tolerate and, in a bad season, these limits may easily be exceeded. Cold or wet weather in spring or early summer, as in 1935, may so retard early growth as to prevent the plant reaching the flowering stage that season. In such circumstances it is impossible to give a figure which will represent the degree of flowering with any certainty.

Number of tillers

The number of tillers was counted in 200 seedlings of common and 200 of giant on 29 July 1937. The seedlings were spaced at 9 in. The results are given in Table III and Graph 7. The number of tillers varied from one to sixteen in giant and from two to twenty-one in common, the most frequent number in the former being six and in the latter nine. The mean number of tillers per plant in giant was 6·67 and in common 8·73, the difference being significant.

Table III

Percentage frequency of tiller numbers in sainfoin seedlings

	% frequency	
No. of tillers	Giant	Common
1	1·5	—
2	1·5	2·0
3	3·0	4·5
4	8·5	3·0
5	15·5	4·5
6	22·0	12·5
7	19·5	12·5
8	14·0	12·0
9	5·0	15·0
10	2·0	9·0
11	1·0	5·5
12	2·5	5·5
13	2·0	2·5
14	—	3·0
15	1·5	2·5
16	0·5	2·0
17	—	1·0
18	—	2·0
19	—	—
20	—	—
21	—	0·5
No. of plants counted	200	200
Mean no. of tillers	6·67	8·73
S.E.	0·1764	0·2482

This greater tillering power in common emphasizes the difference in behaviour between the two varieties. While giant produced tall stems and flowered, common remained prostrate and produced a greater number of tillers.

Discussion

Sainfoin usually is sown with a nurse crop, so that the course of development described above does not necessarily agree exactly with what actually happens in farming practice.

Giant and common sainfoin are taxonomically indistinguishable, and the recognition of a plant as belonging to one or the other variety depends upon growth behaviour. Giant grows more rapidly, producing two or even three cuts per season and is short-lived, while common will only give one cut followed by grazing and is long-lived. The account given above of the behaviour of sainfoin plants in their seeding year is consistent with this distinction, and the difference is exhibited from the beginning. In the first foliage leaf there is no significant difference between the varieties but, in the second to the sixth leaf, giant consistently has a significantly higher average number of leaflets than common. At the six-leaved stage the total number of leaflets averaged 26·9 for giant and 24·0 for common. The surface area of the leaflets was not actually measured, but observation and comparison have revealed no obvious difference between the varieties in this respect. If this be so, then the number of leaflets can be taken as an approximate measure of leaf area, and it follows that in giant the area of each leaf is greater than that of the corresponding leaf of common, and that the rate of increase of leaf area is greater in the giant variety. The rate of increase of number of leaves, however, was the same in both varieties, the appearance of corresponding leaves being almost simultaneous.

At the rosette stage common is more prostrate than giant and, at a later stage, giant tends to run to stem and flower while common retains the rosette habit and produces leaves only. Correlated with this prostrate habit of common is a greater tillering power.

It has been suggested by Koreisa (1935) that the difference in behaviour between the two varieties is due to a difference in the length of the thermo-stage in development. He divides perennial plants into two groups, viz.:

(1) Winter perennials. These are plants which, when sown in spring, do not flower the first season. They have a long thermo-stage and must pass through winter before flowering.

(2) Spring perennials. These plants have a short thermo-stage and, when sown in spring, flower the same season.

Common sainfoin belongs to the first group and giant sainfoin to the second. Temperature may certainly be a factor determining the frequency

of flowering, but to attribute all the differences between the varieties to temperature requirement would be an over-simplification.

The high degree of variability as between individual plants shows that commercial stocks of sainfoin are mixed populations. The frequency curves for numbers of leaflets are very similar in giant and common and there is considerable overlap between the varieties. This suggests that they both have been derived from the same original mixed stock by a process of mass selection. Unfortunately, there is no historical evidence as to the separation of the two varieties.

The distinctive features of giant sainfoin are:

(*a*) In the seeding year it tends to run to stem and flower.
(*b*) Its growth is more rapid and luxuriant throughout.
(*c*) It flowers again after cutting.
(*d*) It is short-lived.

Seed is harvested in the second or third year and this involves a selection in favour of plants which come to maturity quickly and are, therefore, probably short-lived. The seed is always taken from the second cut and consequently only from plants which flower a second time. In comparison with common, therefore, mass selection is for second flowering combined with early maturity.

The distinctive characters of common sainfoin are:

(*a*) In the seeding year the plants are prostrate and no stems or flowers are formed.
(*b*) Its growth is less rapid and luxuriant.
(*c*) No flowers are formed after cutting.
(*d*) It is long-lived.

The position here is complicated by the fact that two regional strains of common have been evolved. In the eastern counties, seed is taken in the early years of a ley, and plants grown from such seed tend to be non-persistent and to show some second flowering. In Hampshire and on the Cotswolds, seed is harvested in the later years of a ley, and gives rise to plants which are long-lived and show no tendency to second flowering (Rees, 1932). Hampshire and Cotswold sainfoin is therefore of the true common type, while Eastern Counties sainfoin is intermediate between giant and true common. Rees shows that there is strong evidence of a correlation between longevity and once-flowering. This would suggest that the original selective factor separating the giant and common varieties from a common stock was the time at which the seed was harvested. By harvesting seed early or late in a ley the two varieties

would become differentiated by the association of flowering frequency and other non-selective characters. It would not be expected, however, that such varieties would be genetically pure and, consequently, there is found considerable overlap between them.

Summary

1. Evidence of delayed germination was obtained.

2. The number of leaflets in each of the first six foliage leaves of sainfoin seedlings shows considerable variation. The mean number of leaflets in each of the second to the sixth foliage leaves is significantly greater in giant sainfoin than in common sainfoin.

3. Giant sainfoin tends to produce erect stems and flower in the seeding year, the amount of flowering being variable. Common sainfoin remains prostrate and never flowers.

4. Common sainfoin produces more tillers than giant sainfoin.

5. The origin of the giant and common varieties is discussed.

I wish to express my thanks to the following seed merchants for supplying samples of seed: Dunn's Farm Seeds, Ltd., Salisbury; Gartons, Ltd., Warrington; A. G. Leighton, Ltd., Whitchurch; Smith Bros., Ltd., Basingstoke; Suttons and Sons, Ltd., Reading; Edward Webb and Sons, Ltd., Stourbridge.

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THOMSON.—The Development of Sainfoin in its Seeding Year (pp. 457–471)

EXPLANATIONS OF PLATES XVIII AND XIX

PLATE XVIII

Fig. 1. Germination of unmilled seeds. $\times \frac{6}{5}$.

Fig. 2. Seedlings at the one-leaf stage with simple and two-foliate leaves. $\times \frac{6}{7}$.

Fig. 3. Seedlings at the two-leaved stage with the combinations 2.3, 1.3 and 1.4. $\times \frac{3}{4}$.

Fig. 4. Seedlings at the three-leaved stage with the combinations 1.3.3 and 1.3.5. $\times \frac{1}{2}$.

Fig. 5. Seedlings at the four-leaved stage with the combinations 1.3.5.5 and 1.3.3.5. $\times \frac{1}{2}$.

Fig. 6. Seedlings at the five-leaved stage with the combinations 1.3.3.5.7 and 1.3.3.3.5. $\times \frac{1}{2}$.

PLATE XIX

Fig. 7. (*a*) Giant seedling with eight leaves and elongated internodes. (*b*) Common seedling at the six-leaved stage. $\times \frac{1}{2}$.

Fig. 8. Common sainfoin, prostrate type. $\times \frac{1}{5}$.

Fig. 9. Common sainfoin, semi-erect type. $\times \frac{1}{4}$.

Fig. 10. Giant sainfoin, semi-erect type. $\times \frac{1}{5}$.

Fig. 11. Giant sainfoin, erect type in flower. $\times \frac{1}{4}$.

(*Received* 14 *January* 1938)

THE ECOLOGY OF THE LARGER FUNGI

II. THE DISTRIBUTION OF THE LARGER FUNGI IN PART OF CHARLTON FOREST, SUSSEX

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(With 1 Text-figure)

CONTENTS

I. Introduction

This investigation was carried out by the two junior authors during the latter part of August and the early part of September 1932. The senior author is responsible for the revision and presentation of the facts in accordance with the general scheme of investigation into the ecological distribution of the larger fungi which is being carried out in this Laboratory. The present work follows the lines indicated in a previous paper by Wilkins *et al.* (1937), and, though this part of the work was completed first, publication has been delayed so that this more restricted but more detailed investigation could be brought into line with the outlook expressed in the previous paper.

II. Experimental method

After a preliminary survey of those parts of Charlton Forest to be investigated, the area was arbitrarily divided into sections (see later) each of which was sufficiently characteristic to merit individual treatment. In each of these sections the phanerogamic vegetation and the fungus flora were examined in detail. The general method was to traverse each section of the area several times, listing what corresponded to a very long zigzag transect. This method gave a random selection of the population in species and frequency. Every fungus was listed and particulars of the surrounding vegetation, as well as of depth of humus and leaf litter, were recorded. The soil texture was determined by digging several small pits in each section and deep pits were dug in certain places. Quadrats and transects were also listed and plotted but these are not included here in detail.

The identification of species was done with the aid of Rea's *British Basidiomycetae* and his nomenclature was followed.

III. Topographical and floristic composition of the area

The area under examination is situated on the summit of the South Downs in Sussex at a height of 600–700 ft. above sea-level. The ridge of

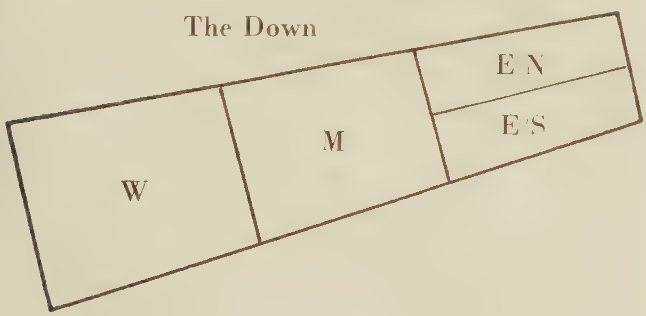

Fig. 1.

the Downs is here covered with thick turf. The steep slope facing north is covered with beech wood of the chalk escarpment type, while the south slope, which has a gradient of about 1 in 100, is clothed with beech wood of the plateau type. The present investigation was confined to part of the latter. The area is roughly rectangular (see Fig. 1), extending from

west to east with its northern boundary where the wood adjoins the Down. It is approximately a mile long and about 300 yards wide in the centre. It is divided into sections as shown, and these sections will be referred to by the symbols given in the figure.

The following details of the floristic composition are not exhaustive since they are intended merely to indicate the main ecological features.

Fagus sylvatica was the dominant tree over the whole area. On the northern edge, where it adjoined the Down, *Quercus robur* and *Fraxinus excelsior* frequently occurred with the beech together with:

Ligustrum vulgare	o.	*Ilex aquifolium*	r.-o.
Viburnum Lantana	o.	*Sorbus aria*	r.

The ground flora of this region consisted dominantly of *Mercurialis perennis* together with: *Asperula odorata*, *Circaea lutetiana*, *Deschampsia caespitosa*, *Oxalis acetosella*, *Quercus* seedlings and *Rubus fruticosus* (agg.).

This type of ground flora extended into the clearings but the frequency of the associated plants varied with the density of the *Mercurialis*. Where the *Mercurialis* was sparse the following were occasional: *Circaea lutetiana*, *Oxalis acetosella*, *Quercus* and *Fraxinus* seedlings, *Rubus fruticosus* (agg.) and various mosses. Where *Mercurialis* was fairly dense the above species became frequent together with the following species which were occasional: *Asperula odorata*, *Deschampsia caespitosa*, *Hedera helix*. In the very densest *Mercurialis*, mosses were rare and no other plants were present.

In the west end of the area (section W) there was a considerable region in which the ground was bare or colonized by sheets of moss. The only plants present besides the beech, which were of spreading form, were a few *Ligustrum vulgare*, very small *Fraxinus* and *Betula* seedlings and here and there a dead ash or birch.

East of this, in section M, the trees were more closely packed and growing to a height of 50–80 ft. Here the ground flora consisted of: *Oxalis acetosella* d., *Deschampsia caespitosa* o.-l.a., *Viola sylvatica* (agg.), o.-f., *Brachypodium sylvaticum*, *Epilobium angustifolium*, *Geranium Robertianum*, *Hedera helix* and *Melica uniflora* o.; with *Rubus fruticosus* (agg.), r.-f., *Asperula odorata* r.-o.; with *Agrimonia Eupatoria*, *Mercurialis perennis* and *Potentilla erecta* r. In other places where trees had been felled or had blown down, regeneration was occurring and young and well-grown specimens of the following were found:

Fraxinus excelsior	f.	*Fagus sylvatica*	r.-o.
Corylus avellana	o.	*Ilex aquifolium*	r.
Quercus robur	o.		

In sections E the ground was more completely dominated by *Oxalis* with *Brachypodium sylvaticum* f., but in the cleared spaces the following were occasional: *Fraxinus* seedlings, *Melica uniflora* and *Rubus fruticosus* (agg.). The dominant extends further into the deep shade than the associates. In the less shaded places where *Oxalis* occurs in moderate density *Deschampsia* is locally frequent, and the occasional species are: *Asperula odorata*, *Epilobium angustifolium*, *Geranium Robertianum*, *Hedera helix*, *Melica uniflora*, *Rubus fruticosus* (agg.) and *Viola riviniana*. More rarely found are: *Agrimonia Eupatorium*, *Mercurialis perennis*, *Potentilla erecta* and some mosses. In the very shaded places the *Oxalis* is sparse and *Deschampsia* is occasional, as are also *Melica*, *Mercurialis* and *Viola*.

The northern part of this eastern section, N/E, is characterized by having extensive dense patches of practically pure *Oxalis* with very rare clumps of *Deschampsia*.

The following species of mosses have been recorded in the ground flora throughout the whole wood: *Mnium hornum* l.a.; *Hypnum cupressiforme* f.; *Catharinia undulata*, *Thuidium tamariscinum* o.; *Hypnum cuspidatum*, *H. molluscum* r.

IV. THE FUNGUS FLORA

A complete list of the species which were found during the investigation is given in Table I. The species are arranged in descending order of frequency, and for convenience of comparison the species are arranged in the same order in all subsequent tables. Column 1 indicates the number of groups and column 2 the number of individuals of each species. The species which were represented by one individual only are collected together at the end.

The outstanding feature of Table I is the small number of species which have a high frequency of individuals and the large number which exist as single specimens. Only seven species were represented by more than 100 individuals, whereas ninety species were represented by less than ten. To some extent this is true of any mycological flora but, in this case, it may have been emphasized by the fact that these fungi had just appeared after the first rain following a very dry summer. It is frequently observed that the beginning of such a season is characterized by profusion of species and paucity of individuals. A somewhat superficial examination of the area later in the season, after the survey was completed, showed at least another twenty species, and a very much greater number of individuals of some of them. This stresses the necessity for due

consideration of time as well as space in relation to fungus distribution. As it is impossible to discuss the distribution of species which are represented by one or two individuals, the subsequent treatment deals only with species which were represented by more than ten individuals.

Table I

Total list of species with frequency of individuals

	1	2		1	2
Marasmius peronatus	57	589	*Lactarius picinus*	9	11
Craterellus cornucopioides	19	538	*Irpex obliquus*	10	11
Marasmius ramealis	36	395	*Androsaceus insititius* var.	6	10
Androsaceus rotula	29	190	*albipes*		
Russula nigricans	34	159	*Lachnea scutellata*	1	10
Collybia fusipes	7	104	*Mutinus caninus*	2	10
Russula foetens	16	104	*Cortinarius cinnamomeus*	5	7
Marasmius dryophilus	15	80	*Lactarius blennius*	5	7
Galera hypnorum	14	78	*Mycena capillaris*	5	7
Russula ochroleuca	36	71	*Androsaceus insititius*	4	6
Marasmius alliaceus	44	69	*Amanita rubescens*	5	5
Lactarius serifluus	12	62	*Armillaria mucida*	2	5
Lycoperdon perlatum	1	50	*Cortinarius leucopus*	3	5
Mycena sanguinolenta	19	48	*Peziza vesiculosa*	2	5
Hydnum repandum	9	46	*Clitocybe infundibuliformis*	1	4
Cantharellus cibarius	15	38	*Russula lutea*	3	4
Polyporus caesius	27	85	*Stereum hirsutum*	4	4
Androsaceus calopus	14	34	*Clavaria rugosa*	3	3
A. epiphyllus	5	30	*Entoloma nidorosum*	2	3
Russula fellea	24	30	*Fistulina hepatica*	1	3
Mycena galopus	9	27	*Lepiota cristata*	3	3
Amanita phalloides	9	26	*Marasmius prasiosmus*	3	3
Russula emetica	22	26	*Mycena acicula*	3	3
Collybia radicata	17	23	*M. galopus* var. *alba*	2	3
Cyathus striatus	4	23	*M. polygramma*	3	3
Cortinarius castaneus	7	19	*Russula fragilis*	3	3
Marasmius globularis	11	18	*Xylaria polymorpha*	1	3
Lactarius acris	9	15	*Collybia butyracea*	2	2
Russula cyanoxantha	15	15	*C. platyphylla*	1	2
R. cutifracta	13	14	*Entoloma sericeum*	2	2
Clavaria cristata	11	13	*Hypholoma fasciculare*	2	2
Cortinarius croceoconus	2	13	*Lycoperdon pyriforme*	2	2
Polystictus versicolor	4	13	*Mycena crocata*	2	2
Lactarius quietus	8	12	*M. pelianthina*	2	2
L. subdulcis	4	12	*M. pura*	2	2
Daedalea quercina	2	11	*Naucoria merismoides*	2	2
Lactarius capsicum	5	11	*Otidea aurantia*	2	2

Androsaceus androsaceus, Amanitopsis fulva, Astrosporina scabella, Auricularia auricula-Judae, Boletus bovinus, B. chrysenteron, Calocera viscosa, Chlorosplenium aeruginosum, Claudopus variabilis, Clitocybe flaccida, Collybia acerbata, Coprinus micaceus, C. plicatilis, Cortinarius cinnamomeus var. *croceus, C. dolabratus, C. paleaceus, C. tophaceus, Daldinia concentrica, Eccilia rhodocylix, Entoloma lividum, Femsjonia luteo-alba, Fomes connatus, F. ferruginosus, Galera spartea, Ganoderma applanatum, Hebeloma crustuliniforma* var. *minus, H. magnimamma, Hydnum cirrhatum, Hygrophorus eburneus, H. russocoriaceus, Inocybe rimosa, Laccaria laccata, Lactarius flavidus, L. uvidus, L. volemus, Lycoperdon echinatum, Marasmius torquescens, Mycena flavo-alba, M. pullata, M. vitilis, Naucoria cerodes, Nolanea proletaria, Omphalia maura, O. pyxidata, Panus stipticus, Phallus impudicus, Pluteus cervinus* var. *Bullii, Polyporus squamosus, Russula atropurpurea* and var. *depallens, R. chloroides, R. coerulea, R. lutea* var. *armeniaca, Stropharia semiglobata, Tricholoma albo-brunneum, T. sejunctum, T. sulphureum, T. terreum, Ustulina vulgaris.*

(*a*) *Distribution in the herbaceous subtypes*

For the purposes of this investigation the area was regarded as consisting of three herbaceous subtypes, viz.:

(i) No field layer.
(ii) *Oxalis* societies.
(iii) *Mercurialis* societies.

Table II

Distribution of fungi in the herbaceous subtypes

	Bare		*Oxalis*			*Mercurialis*		
	Litter	Moss	Sparse	Moderate	Dense	Sparse	Moderate	Dense
Marasmius peronatus	×××××		×××	×××××	×	×××××	××××	××××
Craterellus cornucopioides	××	×××××	.	.	.	.	.	.
Marasmius ramealis	×××××	××××	.	×	.	.	×××	×××××
Androsaceus rotula	×××	×××××	×	×××	.	×××	××××	××××
Russula nigricans	××××	×××××	.	.	.	××	.	.
Collybia fusipes	.	×××××	.	.	.	.	.	××
Russula foetens	××××	××××	×××	.	.	××	.	.
Marasmius dryophilus	××××	×××	.	×××	.	××	×	××
Galera hypnorum	.	×××××	.	××	.	.	.	××
Russula ochroleuca	××	××××	.	.	.	.	.	×
Marasmius alliaceus	×××	××	×	××	×	×××	××	××
Lactarius serifluus	×××××	××	×	×	.	××	×	××
Lycoperdon perlatum	.	×××××	.	.	.	.	.	.
Mycena sanguinolenta	××	××	.	××	.	×	.	×××
Hydnum repandum	×××	×××	.	.	.	.	.	.
Cantharellus cibarius	.	××××	.	.	.	.	.	.
Androsaceus calopus	××	.	.	.	×	××	.	×××
A. epiphyllus	×	.	.	××	.	.	.	×××
Russula fellea	××	×××	×	.	.	.	.	.
Mycena galopus	×	.	.	×××	.	×	.	×××
Amanita phalloides	××	×××	.	.	.	×××	.	.
Russula emetica	××	×××	.	.	.	.	.	.
Collybia radicata	××	××	.	××	.	.	×××	×
Cyathus striatus	.	××	.	.	×	.	.	×××
Cortinarius castaneus	×××	××	.	.	.	.	.	.
Marasmius globularis	.	.	.	×	.	××	×	××
Lactarius acris	××	××	.	.	.	.	.	.
Russula cyanoxantha	××	××	.	×	.	×	.	.
R. cutifracta	××	××	×	.	.	.	.	.
Clavaria cristata	.	×××	.	.	.	×	.	×
Cortinarius croceoconus	.	××	.	.	.	.	.	.
Lactarius quietus	××	×	.	×	.	×	.	.
L. subdulcis	××	.	.	.	.	.	.	.
L. capsicum	××	××	.	.	.	.	.	.
L. picinus	××	××	.	.	.	.	×	.

Table II shows the distribution and relative frequency in the above three types. Each of the subtypes is further divided as shown in the table. Relative frequency is expressed somewhat graphically by the adoption of the following scheme:

××××× = abundant.
×××× = frequent.
××× = occasional.
×× = rare.
× = very rare.

It must be understood that this terminology is adapted to the special case and is purely relative.

A consideration of Table II without reference to the subdivisions of the three subtypes shows that, with the sole exception of *Marasmius peronatus*, no species is equally abundant in all three types. On the other hand, it shows that the majority of species show a marked preference for the areas without field layer. Out of a total of thirty-five species, thirty-four occur on bare ground as compared with eighteen in *Oxalis* and twenty-three in *Mercurialis*, i.e. of the total fungus species all but one are found on those parts which have no field layer, about two-thirds are present in *Mercurialis* and about half in *Oxalis*. The relative frequency of individuals can be estimated by the distribution of the "crosses", if it is assumed that these symbols approximate to a numerical representation of frequency. The total number of crosses in subtype 1 is 161, in 2 it is 44 and in 3 it is 94. It appears, therefore, that relative frequency of individuals expresses the same general idea as does the distribution of the species, but is more pronounced, in that in areas with no field layer there are about twice as many individuals as in *Mercurialis* and about four times as many as in *Oxalis*.

An examination of the subdivisions of the three types brings out the following points:

(i) *Areas without field layer.*

These are divided into regions which are covered with leaf litter and those having a covering of moss. The species which are respectively characteristic of these two regions fall into three groups.

(*a*) Those occurring with almost the same frequency in both, e.g.

Russula nigricans	*Russula emetica*
R. foetens	*Collybia radicata*
Marasmius dryophilus	*Cortinarius castaneus*
M. alliaceus	*Lactarius acris*
Mycena sanguinolenta	*Russula cyanoxantha*
Hydnum repandum	*R. cutifracta*
Russula fellea	*Lactarius capsicum*
Amanita phalloides	*L. picinus*

(*b*) Those relatively frequent in litter but rare in moss, e.g.

Marasmius peronatus	*Lactarius serifluus*

(*c*) Those relatively frequent in moss but rare in litter, e.g.

Craterellus cornucopioides	*Russula ochroleuca*
Collybia fusipes	*Lycoperdon perlatum*
Galera hypnorum	*Cantharellus cibarius*

It has already been mentioned that *Marasmius peronatus* is abundant in all three subtypes. With the exception of this species, the areas with

no field layer are the only ones which contain the larger species of fungi in abundance.

(ii) Oxalis *societies*.

This subtype is again divided according to the density of the herbaceous covering. It is evident that both in number of species and in frequency of individuals it is distinctly poor. No species occurs more frequently in *Oxalis* than in the other subtypes and, except for *Marasmius peronatus*, the only large species which are occasional are *Russula foetens* and *Marasmius dryophilus*. This relative infrequency in *Oxalis* is not a consequence of variation in light intensity, since the average value was found to be approximately the same as in subtype (i). The suggestion that it may be due to competition between fungi and vegetation, resulting from the shallow rhizomatous growth of the *Oxalis*, is rather negatived by the fact (at present unexplained) that the greatest number of individuals was found in "moderate" *Oxalis*.

(iii) Mercurialis *societies*.

This subtype is also divided according to density of the covering. The majority of the commoner species occur in it, but on the whole the species associated with *Mercurialis* are usually the smaller ones. Omitting *Marasmius peronatus* again, only three of the larger forms, e.g. *Marasmius alliaceus*, *Amanita phalloides* and *Collybia radicata*, are found here to the extent of being "occasional". None of these occurs in the "dense" region and it is suggested that the absence of the large fungi may be connected with a possible competition between the fungus mycelium and the rhizomes of the *Mercurialis*, both of which occur at about the same depth in the substrate. The smaller species, on the other hand, with a less deeply seated mycelium do not suffer from this competition, and almost all the smaller species occur in this subtype. *Androsaceus rotula* and *Marasmius ramealis* are of equal frequency here as in subtype (i), while *Androsaceus calopus*, *A. epiphyllus*, *Mycena sanguinolenta* and *Cyathus striatus* are of greater frequency here than anywhere else. In general it seems that the mycological flora of *Mercurialis* consists of the total flora minus most of the larger species. Ramsbottom (1932), writing on the fungi of British beech woods, states: "Where the herbaceous vegetation cover is continuous (*Mercurialis*, *Asperula*, *Rubus*) fungi are scarce, only such small forms as *Mycena galapoda* and occasionally *Paxillus involutus* occurring." With regard to distribution in the subdivisions of this subtype it will be seen that the greatest number of

individuals occurs in the "dense" regions while the "moderate" regions have the least.

Quadrats and transects were laid in these three societies and detailed results were obtained. These confirm the generalizations already stated but are not recorded here in detail.

(*b*) *Distribution in relation to type of substrate*

(i) *Types of soil.*

The soil of the area showed considerable variation. In depth it varied from 10 in. to about 3 ft., and according to the distance of the chalk from the surface its texture varied from moderately heavy clay to a light friable loam. The heavier type of soil was found in the western part (section W in Fig. 1), where clay-with-flints extended to a depth of about 30 in., the flints being very frequent below 15 in. In section M the soil was lighter, more loamy, often more humus-stained, and abounding with earthworms. It was shallow, often not more than a foot deep. In certain places, especially where it had been disturbed, as for instance by tree felling or by burrowing animals, the chalk was apparent on the surface. Section E consisted of a black, earthy loam mixed with many small flints, but here also the chalk sometimes came to within a foot of the surface. At the northern and eastern boundaries chalk was actually present in the surface soil.

It is evident that the soil must show great variability of calcium carbonate content, acidity and texture. The western part was conspicuously lacking in carbonate and had a deep layer of acid humus on the surface, though there was never any sign of leaching or podsolization. The eastern end was characterized by having a soil of a black, friable renzina with carbonate distributed throughout its depth.

From the point of view of fungus distribution it is convenient to arrange the soil types under three headings which, in a general sense, correspond respectively to the three sections W, M and E in Fig. 1. The three soil types are:

(1) Moderately heavy soils, usually with flints which may come to within a few inches of the surface. The pH ranges approximately from 3·9 to 5·4. The soil may be humus-stained, in which case the acidity will be greater.

(2) Light coloured loam soils with flints as in 1. The pH varies from 5·5 to 6·2 and the soil may be humus-stained.

(3) Black, granular, renzina soils which contain flints in varying

quantities throughout, and which differ in virtue of the varying proportions of large chalk particles. The pH range is from 6·4 to 7·2.

Table III shows the distribution and frequency of the relatively abundant fungi in these three types.

Table III

Distribution of fungi in relation to soil types

	(1) Heavy 3·9–5·4	(2) Light 5·2–6·2	(3) Renzina 6·4–7·2
Marasmius peronatus	303	13	271
Craterellus cornucopioides	458	80	—
Marasmius ramealis	60	293	32
Androsaceus rotula	84	78	28
Russula nigricans	59	100	—
Collybia fusipes	—	80	20
Russula foetens	11	81	12
Marasmius dryophilus	—	18	52
Galera hypnorum	63	—	15
Russula ochroleuca	59	8	4
Marasmius alliaceus	19	15	35
Lactarius serifluus	6	53	15
Lycoperdon perlatum	—	50	—
Mycena sanguinolenta	13	20	16
Hydnum repandum	43	3	—
Cantharellus cibarius	19	18	—
Androsaceus calopus	22	—	12
*A. epiphyllus**	—	—	—
Russula fellea	13	7	10
Mycena galopus	1	7	19
Amanita phalloides	9	6	11
Russula emetica	5	19	2
Collybia radicata	14	1	7
Cyathus striatus	2	20	—
Cortinarius castaneus	19	—	—
Marasmius globularis	3	8	7
Lactarius acris	5	10	—
Russula cyanoxantha	4	8	3
R. cutifracta	8	6	—
Clavaria cristata	8	5	—
Cortinarius croceoconus	11	2	—
Lactarius quietus	6	4	2
L. subdulcis	4	6	2
L. capsicum	—	5	6
L. picinus	3	8	—
No. of species	30	31	22
% individuals	45	35	20

* No figures are available for this species.

Table III shows that rather more species were found on the first two types of soil, but, on the whole, species were fairly well distributed throughout all three types. In the case of frequency of individuals, however, there are almost as many in the first type as in both the other two taken together. Few species are restricted to one or other of the soil types. This in fact applies only to one species, *Cortinarius castaneus*,

which is found only on the heavier soil. (*Lycoperdon perlatum* on loam soil is ignored as it occurred only as one group. Table I.) From the standpoint of frequency there may be a distinct preference for one type of soil as shown for instance by *Craterellus cornucopioides*, *Russula nigricans* and *Mycena galopus*, which show a respective preference for types 1, 2 and 3. Some species, such as *Mycena sanguinolenta*, seem to be comparatively tolerant of soil differences.

Large variations in soil characters influence the mycological flora, but it is probable that the small changes in soil texture indicated above do not affect distribution of the fungi to any great extent. In any case a "preference" as suggested by Table III is, most probably, not determined by soil features alone but may be due to type of vegetation, humus, leaf litter, *p*H etc., or to a combination of any or all of these factors. Since the mycelia of fungi usually grow in humus or in leaf litter it was expected that there might be greater correlation between these two substrate factors and fungus distribution.

(ii) *Humus and leaf litter.*

Over most of the area which is not covered by vegetation, the soil is covered with a layer of leaf litter and vegetable debris. The depth of this varies from a few scattered fragments to a dense covering up to 4 in. Below the leaf litter is usually a layer of humus. This is here defined as the upper layer of soil in which incorporation of the vegetable debris with the mineral matter in the soil is still going on. The upper limit of this layer is not sharply demarcated from the overlying leaf litter, but the lower limit is taken to correspond with the absence of decaying organic matter. Apart from parasites, fungi grow on the humus, the leaf litter or on dead wood, and it is convenient to regard the fungus "substrate" from this point of view, i.e. where the mycelium is found growing.

Table IV shows the distribution and relative frequency of fungal species in relation to the three types of substrate, humus, leaf litter and wood. The last mentioned is rather vague as some species on wood may grow on wood either buried in the humus (and could reasonably be transferred to that section) or on or above the surface of the ground.

Table IV shows that by far the greater number of species and the highest proportion of individuals grow in the humus. It is also apparent that there is a tendency towards restriction to one or other of the three sections, and with the possible exception of *Marasmius alliaceus*, even those species which occur in more than one section usually show a preference for one of them.

The distribution of species in relation to humus and leaf litter was investigated further.

Table IV

Distribution of fungal species in relation to humus and leaf litter

	Humus	Leaf litter	Wood
Marasmius peronatus	—	587	—
Craterellus cornucopioides	538	—	—
Marasmius ramealis	—	—	395
Androsaceus rotula	—	3	187
Russula nigricans	159	—	—
Collybia fusipes	—	—	100
Russula foetens	104	—	—
Marasmius dryophilus	—	80	—
Galera hypnorum	78	—	—
Russula ochroleuca	71	—	—
Marasmius alliaceus	14	28	27
Lactarius serifluus	60	14	—
Lycoperdon perlatum	—	—	50
Mycena sanguinolenta	12	35	2
Hydnum repandum	46	—	—
Cantharellus cibarius	37	—	—
Androsaceus calopus	—	34	—
A. epiphyllus	—	9	21
Russula fellea	30	—	—
Mycena galopus	—	27	—
Amanita phalloides	18	8	—
Russula emetica	26	—	—
Collybia radicata	—	—	22
Cyathus striatus	—	—	22
Cortinarius castaneus	19	—	—
Marasmius globularis	—	18	—
Lactarius acris	15	—	—
Russula cyanoxantha	15	—	—
R. cutifracta	14	—	—
Clavaria cristata	13	—	—
Cortinarius croceoconus	13	—	—
Lactarius quietus	12	—	—
L. subdulcis	10	2	—
L. capsicum	11	—	—
L. picinus	11	—	—
No. of species	23	12	9
% individuals	44	28	28

(α) *Humus.* It was shown in Table IV that about one-half the fungi under observation grew in the humus layers of the soil. It has also been stated that this humus may vary in depth from 0 to 2 in. The depth of the humus affects the pH of the surface soil and this factor as well as the presence of food materials may influence fungus distribution. Table V shows the distribution of twenty-five of the more abundant fungi in relation to depth of humus but quite irrespective of the depth of the overlying leaf litter (if any) or of the underlying soil.

Table V

Distribution of fungi in relation to depth of humus

	Depth of humus (in.)				
	0	½	1	1½	2
Marasmius peronatus	—	10	200	253	124
Craterellus cornucopioides	—	71	194	273	—
Marasmius ramealis	14	15	24	340	2
Androsaceus rotula	32	1	52	105	—
5. *Russula nigricans*	—	32	15	50	—
R. foetens	—	15	27	31	31
Marasmius dryophilus	—	35	30	3	12
Galera hypnorum	12	1	2	63	—
Russula ochroleuca	—	14	26	31	—
10. *Marasmius alliaceus*	1	11	28	15	14
Lactarius serifluus	2	33	12	23	4
Mycena sanguinolenta	—	9	9	23	8
Hydnum repandum	—	8	15	20	3
Cantharellus cibarius	—	2	5	13	17
15. *Androsaceus calopus*	—	—	5	27	2
Russula fellea	—	7	5	16	2
Mycena galopus	—	10	3	13	1
Amanita phalloides	—	8	1	6	11
Russula emetica	—	5	4	7	10
20. *Collybia radicata*	—	2	3	13	4
Cortinarius castaneus	—	12	1	6	—
Lactarius acris	—	7	2	2	4
Russula cyanoxantha	1	5	4	2	3
Clavaria cristata	—	7	3	2	—
25. *Lactarius quietus*	—	8	1	2	1
No. of species	6	24	25	25	18
% individuals	2	12	26	49	11

The fungi in Table V are those for which there were sufficient results to show that there was correlation between type of substrate, expressed in terms of humus depth, and their distribution, irrespective of the position of the mycelium.

It appears that few fungi live on ground which is devoid of humus layer. Of those six species which appear in the first column, two, e.g. *Marasmius ramealis* and *Androsaceus rotula*, grow on wood (usually in relation to a humus depth of 1½ in., see col. 4), *Galera hypnorum* grows on moss, and the other three records need not be regarded as significant. Apart from this, species were found to be comparatively evenly distributed in any depth of humus from ½ to 2 in. It seems that a slight covering of humus is almost essential to fungus growth even if the mycelium lives on leaves, e.g. *Marasmius peronatus*, or on wood, e.g. *Marasmius ramealis*. In connexion with frequency it will be seen that about one-half the individuals occurred in a region where there was a humus depth of 1½ in., which seems to be the optimum depth of humus covering.

(β) *Leaf litter.* The leaf litter is made up, for the most part, of beech leaves which are highly resistant to breakdown by micro-organisms and fungi. Not only are these leaves attacked by relatively few fungi, but many fungi, the mycelium of which is found in the broken down humus layers, are actually inhibited by deep layers of leaf litter. On the other hand, there are certain species which are able to grow in humus under a deep layer of leaf litter, but these are probably species which need either comparatively unincorporated humus or else a deep layer of this substance.

Table VI shows the distribution of the same twenty-five species (as in Table V) in relation to leaf litter. This table compares with Table V and, as in the former table, the depth of leaf litter was ignored, so in Table VI the depth of humus is not taken into account.

Table VI

Distribution of fungi in relation to depth of leaf litter

	Depth of leaf litter (in.)				
	0	1	2	3	4
Marasmius peronatus	—	1	30	246	310
Craterellus cornucopioides	538	—	—	—	—
Marasmius ramealis	33	55	16	291	—
Androsaceus rotula	44	6	23	103	14
5. *Russula nigricans*	67	53	29	10	—
R. foetens	33	62	3	6	—
Marasmius dryophilus	4	37	25	4	10
Galera hypnorum	74	1	2	1	—
Russula ochroleuca	18	36	17	—	—
10. *Marasmius alliaceus*	6	29	11	19	4
Lactarius serifluus	4	40	29	1	—
Mycena sanguinolenta	4	7	8	10	20
Hydnum repandum	14	11	21	1	—
Cantharellus cibarius	16	19	2	1	—
15. *Androsaceus calopus*	—	1	6	27	—
Russula fellea	9	13	2	4	1
Mycena galopus	—	—	14	10	—
Amanita phalloides	11	14	1	—	—
Russula emetica	12	8	4	2	—
20. *Collybia radicata*	5	9	1	5	2
Cortinarius castaneus	13	6	—	—	—
Lactarius acris	1	12	1	1	—
Russula cyanoxantha	7	4	3	1	1
Clavaria cristata	10	2	—	—	—
25. *Lactarius quietus*	1	10	1	—	—
No. of species	22	23	22	19	8
% individuals	34	16	9	27	14

Table VI shows that only one of these species, e.g. *Marasmius peronatus*, shows a real preference for deep leaf litter. This fungus has a mycelium which grows in leaf litter (see Table IV), and it seems that the frequency of individuals varies directly with the depth of litter. To a

lesser extent this also applies to *Androsaceus calopus*. The only other fungi which like a deep covering of leaf litter are *Marasmius ramealis* and *Androsaceus rotula*. Both these grow on wood, and it seems reasonable that, if only old and well-decayed twigs are attacked, these are of necessity covered by a deep layer of leaves. At the other extreme is *Craterellus cornucopioides*, which grows on ground which has a covering of moss, and which will only grow where there is no covering of leaf litter at all. The same is also true of *Galera hypnorum*. A definite preference for a very slight leaf covering is also shown by *Russula nigricans*, *R. foetens*, *Cantharellus cibarius*, *Amanita phalloides*, *Cortinarius castaneus* and *Clavaria cristata*.

(iii) *Hydrogen-ion concentration.*

The *p*H of the substrate was determined by means of a B.D.H. Capillator. The *p*H of the soil at 6 in. below the surface varied from 5·9 to 8·2, depending on the amount of chalk present. It has been stated that fungi can be classed as "calcicoles" or "calcifuges" according as to whether they grow on calcareous or on siliceous soils, but there is little agreement between different investigators on this point. This discrepancy is most probably due to the fact that the mycelia of fungi live usually in the humus layers, i.e. in a substrate which is devoid of carbonate and almost always acid. It is doubtful whether the *p*H of the underlying soil has any direct bearing on the *p*H relations of fungi.

Many determinations were made of the *p*H of the humus layers immediately connected with the mycelia of individual fungus sporophores. These results show an even greater variation of *p*H than those of the soil. In the western part of the area where the soil was heavier the *p*H varied from 3·9 to 7·0. East of this the soil became more alkaline, varying from *p*H 5·5 to 7·6. There is often a great difference between the *p*H of the humus layer and that of the underlying soil. In one instance the *p*H of the former was 3·9 while that of the latter 2 in. below was 5·9.

Table VII shows the distribution and frequency of certain fungi in relation to the *p*H values of the substrate in which the mycelium was growing.

From Table VII the approximate range of *p*H toleration and the approximate optimum can be seen. None of the fungi covers the whole range of possible *p*H, though *Androsaceus rotula* covers most of it. Several others show a fairly high degree of frequency over a wide range, e.g. *Marasmius ramealis*, *Russula ochroleuca*, and *Marasmius alliaceus*. Certain species are definitely restricted, as for instance *Marasmius*

Table VII

Distribution and frequency in relation to pH

	3·5–4·0	4·0–4·5	4·5–5·0	5·0–5·5	5·5–6·0	6·0–6·5	6·5–7·0	7·0–7·5	7·5–8·0
Marasmius peronatus	—	199	248	33	—	—	—	—	—
Craterellus cornucopioides	50	210	10	—	196	61	—	—	—
Marasmius ramealis	20	18	19	266	20	15	11	—	—
Androsaceus rotula	—	4	92	—	2	18	57	2	12
Russula nigricans	—	55	57	5	12	9	4	—	—
R. foetens	—	31	31	22	12	—	—	—	—
Marasmius dryophilus	—	39	38	3	—	—	—	—	—
Galera hypnorum	1	1	—	—	4	—	71	1	—
Russula ochroleuca	7	21	7	7	17	5	7	—	—
Marasmius alliaceus	—	17	19	9	4	1	8	7	—
Lactarius serifluus	—	28	1	3	—	30	9	2	—
Mycena sanguinolenta	1	3	22	12	—	5	1	1	—
Hydnum repandum	—	35	3	—	7	—	1	—	—
Cantharellus cibarius	—	5	9	1	12	3	—	—	—
Androsaceus calopus	—	3	8	19	1	—	—	—	—
Russula fellea	—	15	12	1	—	2	—	—	—
Mycena galopus	—	11	5	10	—	—	1	—	—
Amanita phalloides	—	3	11	—	—	1	11	—	—
Russula emetica	—	12	6	4	2	1	1	—	—
Collybia radicata	1	1	1	—	3	—	11	1	—
Cortinarius castaneus	—	—	1	—	8	8	—	—	—
Lactarius acris	—	2	1	7	—	5	—	—	—
Russula cyanoxantha	—	2	4	1	3	2	2	—	—
Clavaria cristata	—	1	4	—	3	2	2	—	—
Lactarius quietus	—	3	—	1	—	—	6	1	—
No. of species	6	24	23	17	16	16	16	7	1
% individuals	3	28	24	16	12	7	8	1	1

peronatus and *M. dryophilus* 4·0–5·5, *Russula foetens* and *Androsaceus calopus* 4·0–6·0. Few show a very decided optimum at any one point and, in any case, the experimental error would negative any definite conclusion. As a generalization it can be said that the optimum for the above fungi appears to be in the region of *p*H 4·0–5·0. The only serious exception to this is *Galera hypnorum* which has a higher optimum at *p*H 6·5–7·0.

V. CONCLUSIONS

It might be well to repeat here a statement made in the previous paper (Wilkins *et al.* 1937) to the effect that this work is suggestive of method rather than conclusive in results. It may tend to anticipate criticism to indicate the main factors responsible for the obvious limitations of the work as expressed in this paper:

(*a*) The fungi recorded are a representative but not a complete concept of the mycological flora of the area.

(*b*) The method of indicating frequency of individuals was purely empirical, and the frequency figures were not relative to any spatial unit.

(*c*) In all the tables the great variation between the numbers of individuals of different species made generalization misleading, as the comparatively high figures for a few species tended to weight the observations.

(*d*) Owing to the small number of species which have a high relative frequency of individuals only a small percentage of the total number of species merited detailed consideration in connexion with distribution.

Nevertheless, within the scope of the investigation, useful information as to the factors which appear to have some direct bearing on the distribution of certain fungal species can be deduced from the results. Coordination of these results has not been attempted, because the data are inadequate, and this would merely lead to inaccurate conclusions. It is suggested, however, that a widespread application of methods similar to those employed here would eventually lead to a more satisfactory knowledge of the relations between fungal species and their habitats. For example, in the case of the first two fungi in the previous lists, particulars can be collected as follows:

(1) *Marasmius peronatus* grew in a plateau beech wood either on a moderately heavy soil with a *p*H of 4·0–6·0, or on a renzina soil with a *p*H of 6·5–7·0. It grew only in deep leaf litter, e.g. about 4 in., overlying a humus layer of about 1–2 in. The essential litter being present, it was equally tolerant of a field layer of *Mercurialis* or of *Oxalis* or of no field layer at all. The mycelium, expressed in terms of sporophore production, appeared to prefer a *p*H in the region of 4·0–5·0. It was abundant from 15 August to 30 September.

(2) *Craterellus cornucopioides* grew in a plateau beech wood showing a distinct preference for a moderately heavy clay-with-flints soil having a *p*H range of approximately 4·0–5·0. It grew only on ground which was devoid of field layer but was invariably associated with a covering of moss. Its fruiting mycelium lived in humus of a depth of 1–1½ in. and appeared to prefer a *p*H of 4·0–6·0. It was abundant from 15 August to 30 September.

The particulars given for the above two species are true under the given circumstances, and it would appear that, if a large number of records somewhat on the lines of the above, well distributed in space and time, could be obtained for all the commoner fungi, the general standard of information as to fungus distribution would be considerably higher and more comprehensive than is at present the case.

VI. SUMMARY

The paper is the second of a series which is dealing with investigations into the ecology of the larger fungi.

It represents a somewhat detailed examination of the fungus flora of a plateau beech wood in relation to (*a*) vegetation and (*b*) substrate.

In the first case, distribution of fungi in the three subtypes—No field layer, *Oxalis* societies and *Mercurialis* societies—is recorded and discussed.

The second section deals with distribution in relation to soil types, humus and leaf litter, with notes on hydrogen-ion concentration.

There is a brief discussion of results.

REFERENCES

The references for this paper are the same as those given in paper I with the following addition:

WILKINS, W. H., ELLIS, E. M. & HARLEY, J. L. (1937). The ecology of the larger fungi. I. Constancy and frequency of fungal species in relation to certain vegetation communities, particularly oak and beech. *Ann. appl. Biol.* **24**, 703.

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BIOLOGY OF OAT SMUTS

V. A TEN YEARS' SURVEY OF SIX SPORE COLLECTIONS. PROPAGATION, SCREENING AND MONOSPORE ISOLATION EXPERIMENTS[1]

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(With Plate XX)

CONTENTS

I. Introduction

In a previous paper (Sampson, 1929), additional evidence of the existence of biological specialization in the oat smut fungi was provided. Using a number of selected oat varieties as differential hosts, spore collections were classified into six types which possessed the characteristic infection capacities shown in Table I. Throughout this paper, the names of species and varieties of oats refer only to line selections having the following reference numbers: *Avena sativa* var. Potato, No. 2855; var. Abundance, No. 2807; var. Grey Winter, No. 2860; *A. nuda*, No. 2495; *A. strigosa*

[1] This paper includes work carried out at the University College of Wales, Aberystwyth, and at the University of Minnesota by the junior author while holding a Ministry of Agriculture Research Scholarship (1933–6). It is embodied in a thesis presented for the degree of Ph.D. of the University of Wales.

orcadensis, No. 521; *A. brevis*, No. 2384. These selections have been multiplied every year by growing a few plants in pots at a distance from other oats. The propagation of the smuts on appropriate varieties is described later. Collection C_3, a duplicate of C_4, was discarded at an early date, and collection L_1 was lost after a few years, probably as a result of contamination by L_2. Since both were propagated on var. Potato the accident was not discovered until it was too late to trace its source and it is impossible to recover L_1 by screening. Fortunately L_{12}, a duplicate of L_1, had been kept in cultivation. Thus the six types recognized as distinct biological species in 1927 have been grown and studied for a period of 10 years, and additional information has been obtained concerning their behaviour and relative stability on the differential hosts.

In work with smut fungi the terms "biological species" or "physiologic race" have been applied, in most cases, to spore collections which have given distinctive and consistent results over a number of years. One of the authors used the term biological species in this sense in 1929 although recognizing (Sampson, 1929, p. 78) "that certain collections (e.g. L_2) which produce infection on varieties belonging to widely different species of the host may be a mixture of two or more biological species". In other words, a so-called biological species may be a population of distinct biotypes, which gives consistent results over a certain period of time. Ideally a biological species should consist of only one genotype. Following Resolution 14 of the Sixth International Congress, Amsterdam, 1935, it is proposed in future to substitute "physiologic race" for "biological species" used in earlier papers of this series.

There are three methods of treating spore collections of smut fungi to bring them nearer to the desired purity of type. One is the use of differential host varieties in screening experiments. Given a mixture of spores of two types such as L_{11} and L_{12} (Table I) it should be possible, *if hybridization does not take place*, to obtain a clear cut separation in *one* generation by dusting the grain of the two differential hosts *strigosa* and Potato. Each host is completely uncongenial to one of the smut types and screens it out of the mixture. If the two smuts hybridize freely and segregate on Mendelian lines, the effect of screening upon a mixed population will depend largely upon the behaviour of the heterozygous dikaryophyte. Even so, we might expect that screening would, in the long run, isolate types closely approaching the two parents. For further discussion on the possibility of natural hybridization in the oat smut fungi see p. 502 of this paper.

Table I

The behaviour of eight spore collections of oat smuts on selected differential hosts

Compiled from data published by Sampson (1929, Tables IV, V and VI) and from additional data. See also Sampson (1933, Table I)

	Ustilago Avenae (Pers.) Jens.			*Ustilago Kolleri* Wille*		
	L_1 and L_{12} Wales	L_2 U.S.A.	L_{11} Wales	C_1 Wales	C_2 U.S.A.	C_3 and C_4 England
A. sativa:						
Potato	+	+	0	0	+	+
Abundance	0	+	0	Not used for *U. Kolleri*		
Grey Winter	Not used for *U. Avenae*			0	+	+
A. nuda	0	+	0	0	+	S
A. strigosa orcadensis	0	S	+	+	+	0
A. brevis	0	0	+	+	0	0

0 = no smutted panicles.
+ = completely susceptible, usually 90–100%.
S = slightly susceptible 15–30%.

* The specific name *U. Kolleri* is now adopted in place of *U. laevis* used in previous publications in conformity with the list published by the Plant Pathology Subcommittee of the British Mycological Society (*Trans. Brit. Mycol. Soc.* **14**, 140 (1929)).

The second method of purifying material is to isolate a single chlamydospore, grow it in culture and build up a collection of spores by inoculating seedlings of a suitable host. Theoretically this procedure does not of necessity lead to a genetically pure strain of the fungus since the parasitic phase of the pathogen is dikaryophytic, nuclear fusion occurring in the chlamydospore, segregation in the promycelium, while sporidial or hyphal fusion with the pairing of nuclei precedes invasion of the host

Table II

Hosts on which the spore collections were propagated, 1928–35

One year (1929) was missed, and in 1930 one-year-old spores were used. Since 1935 monospore lines have replaced the original spore collections

	Spore collections					
	Ustilago Avenae			*Ustilago Kolleri*		
Year	L_2	L_{11}	L_{12}	C_1	C_2	C_4
1928	Potato	*Strigosa*	Potato	*Strigosa*	*Nuda*	Grey Winter
1930	,,	,,	,,	,,	Potato	,,
1931	,,	,,	,,	,,	*Strigosa*	Potato
1932	*Nuda*	,,	,,	,,	,,	,,
1933	,,	,,	,,	,,	Potato	,,
1934	,,	,,	,,	,,	,,	,,
1935	,,	,,	,,	,,	,,	,,

(Dickinson, 1927, 1927–8; Popp & Hanna, 1935). The purity of the population ultimately arising from a single spore will depend, therefore, upon the genetical constitution of the chlamydospore selected. A third method which should give quicker results is to start the cultures from the paired sporidia of one chlamydospore.

The first two methods have been studied at Aberystwyth. The object of this report is to re-examine the pathogenicity of the 1927 spore collections in the light of these results and to see in what degree they approached pure-breeding populations.*

II. TECHNIQUE

(1) *Propagation of spore collections*

Each year since 1927 the spore collections have been propagated upon appropriate hosts, using the following technique. Shelled grains of each oat selection were dusted with spores, placed in rows between two sheets of damp blotting paper, which were made into rolls and placed in an incubator at 22° C. for 3 days. The seedlings were transferred to boxes of soil (usually heated to destroy stray grains of oats and weeds) and left in a cool glasshouse for some weeks. Later the boxes were placed in a trench in the open cage until smutted panicles were formed in the sheath. Before exsertion took place the boxes were taken back to the glasshouse and glazed bags were tied firmly over the stems so that the spores matured in the bag. When the plants were judged to be ripe the stems were cut, tied in bundles without removing the bags and stored in the laboratory. During the winter the spores were rubbed through fine sieves and transferred to clean jars. Two collections were never dealt with in the same room on the same day, and, at each step of the work, precautions were taken to prevent admixture of strains. It was not practicable to keep each collection in a separate glasshouse.

Apart from the loss of collection L_1, the technique proved satisfactory. The susceptible varieties chosen as hosts (Table II) invariably gave 80–100% infection, and two boxes of each lot, comprising 60–80 plants, yielded an adequate supply of spores for the experiments involved.

(2) *Screening experiments*

The necessary annual propagation may change a spore collection by sifting out certain types. In experiments particularly designed to test the effect of screening, a portion of the spore collection was used for the

* Only pathogenicity is dealt with. Some of the collections of *U. Kolleri* contain echinulate spores but this does not affect the argument of this paper.

inoculation of the selected variety, spores were harvested under a new reference number and grown on the same variety for several years in succession. Finally, a test was arranged by which the "new" collection could be compared with the "parent" collection on several differential hosts. The method is essentially like that followed by other workers (Dillon Weston, 1932; Nicolaisen, 1934).

(3) *Monospore lines*

Isolation of spores was undertaken in February 1934, using a modification of the method described by Dickinson (1926). The procedure involved the removal of dry spores from a glass slip and their transference to agar drops on sterile cover-slips. The whole of this process, and the subsequent germination and development of the young colonies, could be kept under direct observation, and all contaminations were immediately discarded. The young colonies were later removed to tubes or plates and kept until required.

In accordance with the experience of several other workers, germination of isolated chlamydospores was, at first, found to be poor, even though others from the same collections germinated and grew readily on ordinary dilution plates. Attempts were made to pick off spores in tap and distilled water, very dilute agar, and cane-sugar solutions of varying strengths, with very indifferent success, and it was not until a dilute solution of sodium carbonate was tried that satisfactory germination was secured.

Stock cultures were grown on 1 % meat extract agar, but since a weak medium, namely, 0·3 % Knop solution agar, was found particularly favourable for sporidial fusions, this was the medium used for inoculation experiments. Shelled grains of the host varieties were germinated on filter paper, and after 2 days, when the plumules were just commencing to grow, they were rubbed with sporidia from a culture which had been well mixed in order to increase the possibility of including sporidia of both sexes. A small piece of agar, bearing sporidia, was then cut out and left attached to the young seedlings, after which the grains were planted in moist soil in earthenware dishes, covered with small bell-jars to maintain a high humidity, and placed in an incubator at 22° C. The following day the process was repeated and the dishes left in the incubator for several days and then removed to window ledges at room temperature. When the seedlings were from 2 to 3 weeks old they were transplanted to pots and boxes in a cool glasshouse for further development. The method was tedious but gave fairly satisfactory results; 53 %

of the total number of plants was smutted, and only four lines out of a total of thirty failed to reproduce spores. The electrical vacuum-pump method of inoculation described by Allison (1937) was also used successfully in some experiments. The chlamydospores obtained were tested in 1935 in the differential host varieties by the technique outlined above (p. 493), and additional confirmatory tests were made in 1936.

Since difficulty was experienced in obtaining germination of isolated single spores of C_4, a slight modification in technique was necessary for this collection. Since good germination was observed when the spores occurred on the agar in clusters, spores for isolation were smeared along one side of an agar drop and removed when the first indication of a promycelium could be seen. Subsequent growth was normal and by this method eight lines were obtained and studied.

(4) *Comparative growth of monospore lines in culture*

Having obtained several monospore lines of each spore collection in culture, experiments were designed to compare their cultural characteristics. The medium selected was similar to that used by Dickinson (1928). Its composition was as follows:

	gm.
Maltose	0·536
Urea	0·011
Dipotassium phosphate	0·500
Magnesium sulphate	0·250
Potassium chloride	0·250
Agar	1·500

Distilled water to make up 100 c.c.

Dipotassium phosphate was preferred to the acid phosphate used by Dickinson, and the *p*H was adjusted to 5·6 by means of dilute hydrochloric acid. All the cultures used for comparison were grown in dishes of the same size, and 7·5 c.c. of medium were introduced into each. This amount ensured an adequate supply of nutrient and, at the same time, left a relatively thin film of agar on the bottom of the container through which the details of growth and development could easily be studied. All cultures were grown at room temperature, and experiments designed to establish the presence or absence of individual characteristics of the spore collections, and the amount of variance between monospore lines within each one of them, were carried on for some months. As these were repeated several times and, where possible, set up in triplicate, it is considered that the results obtained present a true picture of the cultural behaviour of the different lines studied.

III. Presentation of results

(1) *Pathogenicity of the spore collections*

It is convenient to discuss each collection separately, recording only changes which may have taken place in the course of propagation or in connexion with the isolation and multiplication of monospore lines.

Collection L_2 was marked in 1927 by a wide range of infection attacking a number of *sativa* varieties and in addition selections of *nuda* and *strigosa*. L_2 is an American collection, and similar results were obtained with it at Missouri (Reed, 1929). Since 1927 it has been propagated either on Potato or *nuda* (Table II). Subsequent tests have shown that its virulence on these hosts is unchanged, that is, it is apparently not a mixture which can be separated into two types by propagation on these hosts. On the other hand, it has lost the power to infect *strigosa*. Five monospore lines were studied and they gave uniform results attacking Potato, Abundance and *nuda*, and failing on *strigosa* and *brevis* (Table III).

Table III

Inoculation of differential hosts with monospore lines, 1935–6. Ustilago Avenae

Ref. to monospore line	% infection: Potato (2855)		Abundance (2807)		*A. nuda* (2495)		*A. strigosa* (521)		*A. brevis* (2384)	
	1935	1936	1935	1936	1935	1936	1935	1936	1935	1936
$L_{2/1}$*	17	—	4	—	15	—	0	—	—	—
$L_{2/2}$*	42	—	30	—	27	—	0	—	—	—
$L_{2/3}$	78	—	64	—	69	—	0	—	—	—
$L_{2/4}$	76	97	92	67	83	98	0	0	—	0
$L_{2/5}$	81	68	74	58	96	95	—	0	—	0
$L_{11/1}$	0	—	0	—	0	—	100	—	—	—
$L_{11/3}$	0	0	0	0	0	0	100	100	—	100
$L_{11/4}$	0	—	0	—	0	—	62	—	—	—
$L_{11/5}$	0	—	0	—	0	—	88	—	—	—
$L_{12/1}$	—	98	—	0	—	0	—	0	—	0
$L_{12/2}$	—	100	—	0	—	0	—	0	—	0
$L_{12/3}$	—	97	—	0	—	0	—	0	—	0
$L_{12/5}$	—	58	—	0	—	0	—	0	—	0

* Low infection with these lines was due to loss of viability in the chlamydospores.

Collection L_{11} has a limited host range attacking only selections of *strigosa* and *brevis* (Sampson, 1929, Tables IV and V). Repeated propagation on *strigosa* (at least 7 years) has not affected adversely its virulence on *brevis*, and four monospore isolations gave uniform and similar results (Table III). Further reference to the apparent purity of

this type will be found in connexion with the growth of monospore lines in culture (p. 501).

Collection L_{12} was propagated consistently on Potato. Four monospore isolations were made in 1935, multiplied on Potato and tested on five hosts in 1936 (Table III). Only Potato was infected, and the monospore lines differed consistently from L_{11} and L_2, and agreed with the original (1927) collection L_1 and the parent collection L_{12} in their inability to attack Abundance, *nuda*, *strigosa* and *brevis*.

Collection C_1. In regard to its infection capacity, this collection of *Ustilago Kolleri* is similar to *U. Avenae* L_{11}, attacking only *strigosa* and *brevis*. Five monospore lines gave uniform results (Table IV) infecting both *brevis* and *strigosa* heavily, although the parent collection had been propagated for six successive generations exclusively on the latter host.

Table IV

Inoculation of differential hosts with monospore lines,
1935–6. Ustilago Kolleri

Ref. to monospore line	% infection									
	Potato (2855)		Grey Winter (2860)		*A. nuda* (2495)		*A. strigosa* (521)		*A. brevis* (2384)	
	1935	1936	1935	1936	1935	1936	1935	1936	1935	1936
$C_{1/1}$	0	0	—	0	—	0	100	100	100	100
$C_{1/2}$	0	—	—	—	—	—	100	—	100	—
$C_{1/3}$	0	—	—	—	—	—	100	—	100	—
$C_{1/4}$	0	—	—	—	—	—	100	—	100	
$C_{1/5}$	0	—	—	—	—	—	89	—	68	—
$C_{2/2}$	89	96	—	34	—	89	95	99	0	0
$C_{2/3}$	100	79	—	41	—	77	100	98	0	1
$C_{2/5}$	93	95	—	71	—	90	0	0	0	0
$C_{4/1}$	75	100	—	84	0	0	0	0	0	0
$C_{4/2}$	80	100	—	82	70	100	0	0	0	0
$C_{4/3}$	83	96	—	50	96	100	0	0	0	0
$C_{4/5}$	75	98	—	78	91	93	0	0	0	0
$C_{4/8}$	—	93	—	—	—	0	—	—	—	
$C_{4/12}$	—	93	—	—	—	0	—	—	—	—
$C_{4/13}$	—	93	—	—	—	0	—	—	—	

Collection C_2, like the original L_2, attacked *strigosa*, *nuda*, and several varieties of *sativa* (Table I). It has been propagated on the three species since 1927 without any apparent screening effect. Of three monospore isolations made in 1934, two were uniform in behaviour and had the wide infection capacity of the parent collection, the third attacked Potato and *nuda*, but failed to attack *strigosa*. Since the infection experiment was repeated in 1936 with fully confirmatory results there seems to be little doubt that the monospore line $C_{2/5}$ is completely lacking in the power to infect *strigosa*. The significance of this result will be discussed

later in connexion with some isolations from C_4 which showed a somewhat similar change.

Collections C_3 *and* C_4[1] came originally from England on Grey Winter, and on this variety it has continued to give high infection. It also infects Potato with equal ease, and growth on either variety fails to change its virulence for the other host. In 1927 it seemed to have the property of infecting *nuda* to some extent. Including its duplicate C_3, five results were obtained in which an attack of 15–28% was recorded on *nuda*, and it became a matter of interest to know if it was a constant character of this collection to give a positive infection of this magnitude on *nuda*. If not, it might be expected that the degree of infection could be increased by growing for successive years on this host. The results shown in Table V were obtained.

Table V

Screening experiment with C_4 *on* Nuda

1930	1931	1932	1933	1934	1935*
% smut grown and harvested repeatedly on *nuda*					
15	15	7	10	0	0
Smut grown on potato 1930–2 then tested on *nuda*					
—	—	9	7	0	0

* In 1935 it was necessary to use 1933 spores owing to failure on *nuda* in 1934.

The implication from such an experiment is that the power to produce slight infection on *nuda* is a fairly stable character in collection C_4, since the infection during the years 1927–33 ranged from 7 to 28%, and no sudden change resulted from screening. The results with monospore lines were entirely at variance with such a conclusion. The seven monospore lines isolated in 1933 and tested on both Potato and *nuda* fall into two distinct groups (Table IV). $C_{4/2}$, $C_{4/3}$ and $C_{4/5}$ gave full infection on both hosts, while $C_{4/1}$, $C_{4/8}$, $C_{4/12}$, and $C_{4/13}$ infected only Potato. The data have been examined, and it is impossible to explain them by the presence of chance impurities in either host or fungus. It is evident that the infection of *nuda* by collection C_4 is due to the presence of the potato-*nuda* type which was revealed when monospore isolations were studied but, even allowing for hybridization between the two types, it seems odd that screening on *nuda* failed to change the degree of infection on that host. Further light may be thrown on the problem by a study of monospore isolations from those lines which infected both the hosts and from the behaviour of the two types in mixture.

[1] These were regarded as duplicates and only C_4 was maintained in cultivation after 1927.

A summary of the behaviour of all the spore collections is given in Table VI which shows the salient features of the monospore lines at present in cultivation.

Table VI

Behaviour of six collections of oat smut over a 10-year period of propagation involving both screening and the isolation of monospore lines

Ustilago Avenae

	L_2	L_{11}	L_{12}
1927	Potato Abundance *nuda* *strigosa*	*strigosa* *brevis*	Potato
1937	Lost by screening the capacity to infect *strigosa*	No change	No change

Ustilago Kolleri

	C_1	C_2		C_4	
1927	*strigosa* *brevis*	Potato *nuda* *strigosa*		Potato *nuda* (slight)	
1937	No change	No change	Lost capacity to attack *strigosa*	Potato *nuda* (100 %)	Potato

(2) *Cultural characteristics of monospore lines*

Dickinson (1928) and Holton (1932) studied the cultural characteristics of monosporidial lines and showed that they segregate in a regular manner on the germination of the chlamydospores. A colony derived from a single chlamydospore may, therefore, represent not a single phenotype, but a population of haploid mycelia of different types. It might be expected that the characteristics of the different types would mask each other and that the features of a monospore colony would be more difficult to define and less constant in subculture than those of a monosporidial colony. It would not be surprising if false sectoring were a common phenomenon arising from the predominating growth of a particular type at one side of the colony. In point of fact sectoring was absent in the monospore colonies, and they showed rather well defined characteristics which reappeared with somewhat unexpected regularity in subsequent transfers. That these features were a composite effect and that segregation for cultural characteristics did occur in the promycelium was shown by a comparison of monospore and monosporidial cultures

Table VII

The chief cultural characteristics of six spore collections. Description based in each case on five monospore lines. Age of colonies 45 days

Ref. to collection	Centre of colony: Colour	Centre of colony: Type	Margin of colony: Colour	Margin of colony: Type	Range in diameter in mm.
C_1	Brown	Slightly raised with central depression. Glossy	Greyish brown	Regularly circular. Inconspicuously zonate	43–45
C_2	Yellow changing to brown with age	Raised, small, with an eccentric depression	Greyish white	Dense white median band. Peripheral radial striations	29–33
C_4	Buff to brown	Raised with a regular "double centre"	White	Sharply zonate with radiating hyphae at periphery	36–43
L_2	Brown	Slightly raised, small, glossy	Greyish brown	Closely zonate, some cultures with well defined lighter bands. Radial striations	38–46
L_{11}	White	Large, convoluted	Opaque milky white	Zonate with pronounced bands. Lobate margins	36–43
L_{12}	Brownish	Small, slightly convoluted. Glossy	Greyish brown	Indistinct zonation. Dull coloured broader bands in some cultures	42–46

from one collection (L_2). There is no doubt that monosporidial isolates are essential for a precise study of cultural features in the smut fungi, but the following observations concerning the growth on an artificial medium of five monospore lines obtained from each of the spore collections under discussion are not without interest. The salient features of each collection are summarized in Table VII. Familiarity with the cultures made it possible to recognize individual peculiarities such as width of zonation which reappeared consistently in parallel transfers, but they defy exact description and it would serve no useful purpose to dwell on them here. The facts which call for emphasis are as follows: (1) The absence of any distinguishing cultural characteristics between the taxonomic species[1] *Ustilago Avenae* and *U. Kolleri*. A similar result was recorded by Rodenhiser (1928). (2) The absence of any correlation between pathogenicity and growth in culture. For example, collections L_{11} and C_1 possess quite different cultural characteristics and monospore

[1] These might with justice be regarded as echinulate and smooth spored varieties of the same species.

line $C_{2/5}$ did not show a closer resemblance to $C_{4/2}$ than did the other C_2 lines (see Table IV for pathogenicity). (3) The extraordinary uniformity of monospore cultures within the collection L_{11}. The white convoluted centre and opaque milky white margin made it possible for any observer to pick out any one of the five monospore lines of L_{11} and the distinction was not lost in old cultures or in subcultures. Moreover, when this collection was selected for a study of monosporidial lines, they showed, in contrast with those of L_2, almost perfect uniformity, indicating the complete absence of segregation in any characteristic which could readily be defined (Pl. XX).

(3) *The stability of resistance to smut in oats*

The resistance of an oat variety to a particular collection of smut spores is measured normally by the percentage of plants which carry smutted panicles when shelled grains are heavily dusted with spores and sown under optimum conditions for infection. The degree of infection in a highly susceptible variety depends largely upon the environment of the grain at the time of germination, a low moisture content favouring infection (Sampson, 1929). Little information is available concerning the later effect of environment on the development of smut. This is a matter of particular interest, as so-called resistant varieties may carry mycelium in their basal tissues even until maturity, without the production of smutted panicles. Can this resistance be changed by any method, or do host and parasite make the same response to an altered environment?

Experiments designed to answer this question have been made at Aberystwyth and are summarized below.

In 1925, starting with somewhat poor soil, artificial manures were used to improve the growth of the crop. Phosphates in particular gave very high increases in yield, but failed to influence the percentage of smutted plants.

For two seasons (1927–8) pot experiments were conducted in which growth was increased or reduced by varying both the moisture of the soil and the nitrogen supply. Tillering in particular varied widely in the different series giving a range per plant of 1·8–4·4 in *nuda* and 1·9–6·0 in Record, but there was no correlation between this or any other aspect of growth and the development of smut.

In 1933–4 an extensive experiment was set up using shelled grains of *strigosa* contaminated with spores of collection C_4 to ascertain if smutted panicles could be induced by cutting back the plants after different

intervals of time. Germination was carried out under conditions which are known to allow the smut to enter the plant (Sampson, 1933). In no case did the removal of the first-formed tillers influence the degree of visible infection; a similar result was obtained by Western in other experiments.

Additional attempts to break down the resistance of a variety were made in 1934, using another collection (L_2) which invades var. *strigosa* but fails to produce spores. Hypodermic inoculation of the growing points of young oat seedlings was carried out with (1) a suspension of spores, and (2) a suspension of two monosporidial lines of opposite sex in distilled water. In each case the result was negative.

In another experiment grains dusted with the spores of L_2 were grown for 3 days under optimum conditions for infection and then kept at a low temperature (2 and 5° C.) for 4 weeks. Growth was checked but, subsequently, the plants matured normally producing healthy panicles.

It is concluded that it is not easy to break down the resistance of oats to smut though, admittedly, there are other methods such as the use of narcotics and high temperatures which have not been tried. Little experimental work has been done on the possibility of changing the relationship in the direction of increased resistance.

IV. Discussion

Although cytological evidence is still scanty, results have been obtained during the past 20 years which establish on a firmer basis the supposition that segregation on Mendelian lines takes place in the fungi.

So far as we know at present, the oat smuts are non-pathogenic in the haploid condition, but the invading parasitic mycelium may be derived either from a pair of fused sporidia or other functional gametes, or from the promycelium which is the immediate result of germination in the chlamydospore. Western (1937) obtained evidence which suggests that the latter method is the one which happens most frequently under natural conditions, and this is a point which bears directly on the behaviour of different races in mixture. To take an extreme case, it seems as if it would be possible theoretically for the heterozygous condition to persist through a number of chlamydospore generations, and it is probably this which limits the efficiency of screening experiments as a means of obtaining races genetically pure for pathogenicity characters.

The persistency of the heterozygous condition seems to be the most likely explanation for some of the facts presented in this paper. From

collection C_2 two types were obtained (in 1933) in the monospore lines; type (*a*) behaved like the parent collection in that it infected Potato, *nuda* and *strigosa*, while type (*b*) infected only the first two hosts. In 1931 and in 1932 collection C_2 was grown on *strigosa*, and one might have expected that this would have screened out type (*b*) if it existed as such in the original collection. The implication is that type (*a*) probably represents the 1927 collection, and that it is segregating for the capacity to infect *strigosa*. It is not yet known in what manner or how frequently this segregation takes place, since data on later generations are still incomplete.

Collection C_4 also yielded two types, but in this case both differed from the parent collection, the one infecting *nuda* more completely and the other giving no smutted panicles on *nuda*. The consistently positive but low infection obtained with the parent collection for a number of years must, in some way, be related to the presence of these types in the 1927 collection, yet it is obvious they could not have been there as a mechanical mixture. This would seem to be another example of the persistency of the heterozygous condition through several generations of chlamydospores produced in the host plant, but data are not yet available for a satisfactory explanation of the behaviour of collection C_4 and its component types. Nicolaisen (1934), working with a number of different spore collections, concluded that they represented a number of distinct biotypes which were heterozygotic for both cultural and pathogenic characters.

Another aspect of the results which is worthy of emphasis is the tendency to obtain a clear-cut distinction between susceptibility and resistance. The authors have not yet found a pure-breeding line which has the capacity of producing consistently a low infection upon a certain oat variety. This is in agreement with the sharply delimited grades of resistance already described in histological studies (Sampson, 1933; Western, 1936).

V. SUMMARY

1. An attempt has been made to trace the history of six spore collections of the oat smuts during a 10-year period of experimental work and to compare them in regard to stability.[1]

2. Evidence of change was found in three collections and the modifications, with one exception, were in a negative direction.

3. At an early stage in the work, collection L_2 lost by screening the capacity to attack *strigosa*.

[1] See footnote p. 493.

4. By monospore isolations, collection C_2 was resolved into two types, one of which had a narrower range of infection than the parent collection.

5. Collection C_4 also yielded two types, but both differed from the original collection. One was more pathogenic and the other less pathogenic than the collection as a whole.

6. Reasons are given for the view that the changes were due to heterozygous types in the collections.

7. No changes were detected in three collections.

8. One collection (L_{11}) was outstanding in the uniformity of monospore and monosporidial lines in culture.

9. Experiments designed to break down the resistance to smut in certain selected oat varieties met with no success.

VI. Acknowledgements

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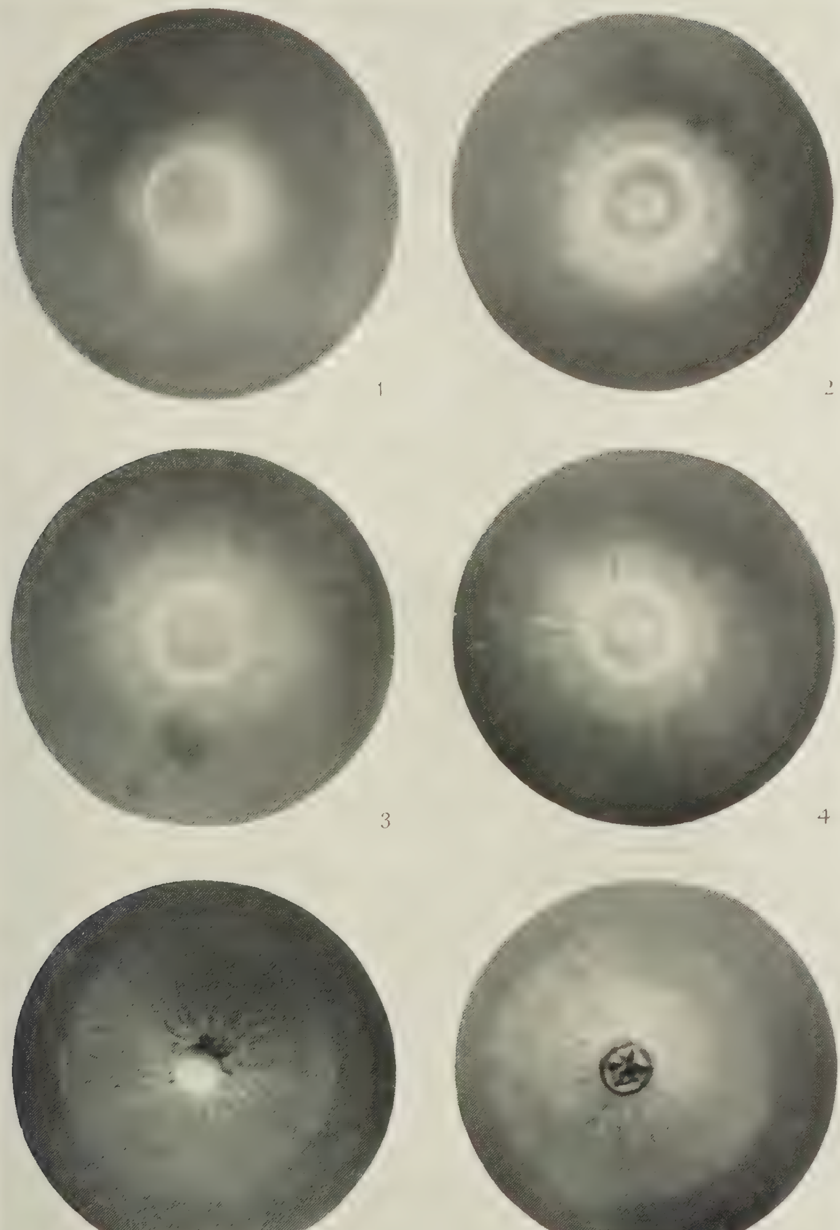

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EXPLANATION OF PLATE XX

The authors are indebted to Mr D. Walters Davies, B.Sc., for assistance in photographing the cultures.

Figs. 1–4. Four monosporidial lines from the four segments of the promycelium of a single chlamydospore of L_{11}, showing no evidence of segregation for cultural characteristics.

Figs. 5–6. Two contrasting monosporidial lines from a single chlamydospore of L_2.

Colonies 45 days old on artificial medium (see p. 501).

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A STUDY OF CROWN RUST, *PUCCINIA CORONATA* CORDA, IN GREAT BRITAIN

II. THE AECIDIAL HOSTS OF *P. CORONATA*[1]

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(With Plate XXI and 1 Text-figure)

CONTENTS

Introduction

In a previous paper (Brown, 1937), it was reported that the following varieties of *Puccinia coronata* Corda could be differentiated in Great Britain by their pathogenicity in the uredospore stage:

(1) var. *alopecuri* infecting *Alopecurus pratensis*,

(2) var. *arrhenatheri* infecting *Arrhenatherum avenaceum*,

(3) var. *avenae* infecting *Avena* spp.,

(4) var. *calamagrostidis* infecting *Calamagrostis lanceolata* and *Phalaris arundinacea*,

(5) var. *festucae* infecting *Festuca elatior*,

(6) var. *holci* infecting *Holcus lanatus*,

(7) var. *lolii* infecting *Lolium perenne*.

The present paper records experiments to determine the relationship between these varieties and the two species of *Rhamnus*, *R. Frangula* and *R. cathartica*, which are the aecidial hosts of the fungus in this country.

[1] This work was carried out from 1932 to 1934 at the Cambridge University Botany School and from 1934 to 1935 at the Dominion Rust Research Laboratory, Winnipeg, Canada.

Klebahn (1892–1912), Eriksson (1894–1909) and Mühlethaler (1910, 1911) found that the varieties of crown rust obtained in Europe had their aecidial stage either on *R. Frangula* or on *R. cathartica*, but not on both. For this reason Klebahn (1892) divided the original species *Puccinia coronata* Corda into two, retaining the name *P. coronata* for those varieties with their aecidial stage on *Rhamnus Frangula*, and giving the name *Puccinia coronifera* to those with their aecidial stage on *Rhamnus cathartica*. The varieties *calamagrostidis* and *agrostidis* were included in the former species and the varieties *avenae*, *alopecuri*, *festucae*, *lolii*, *glyceriae* and *epigaei* in the latter. As rust from *Holcus* spp. and *Agropyron repens* appeared sometimes to infect *Rhamnus Frangula* and sometimes *R. cathartica*, it was considered that these grasses were infected by varieties of rust belonging to both species.

Treboux (1912), working in Russia, found that the supposedly "*coronata*" hosts, *Calamagrostis lanceolata*, *Phalaris arundinacea* and *Agrostis stolonifera*, were sometimes infected by aecidiospores from *Rhamnus cathartica*, and at a later date (1914) he found that the "coronifera" host, oats, could be slightly infected with aecidiospores from *R. Frangula*. He considered that the distinction between the two species was not as clearly cut as had been supposed by Klebahn and Eriksson.

A similar conclusion was reached by Melhus *et al.* (1922) and Dietz (1926*a*), who investigated the aecidial hosts of *Puccinia coronata* in the United States. They inoculated a number of *Rhamnus* species with sporidia from teleutospores on oats, *Calamagrostis canadensis* and *Festuca elatior*, and found no marked difference in the alternate host range of rust from these grasses. *Rhamnus cathartica* and other closely related species could be infected heavily by teleutospores from all three hosts, but the spores on oats and *Calamagrostis canadensis* also caused slight infection of *Rhamnus Frangula*. It appeared, therefore, that Klebahn's division of *Puccinia coronata* Corda into two species did not hold for American material.

In 1931, four pathogenic types of crown rust were distinguished in Canada by Fraser & Ledingham (1933). The aecidial stages of these forms occurred on *Rhamnus cathartica*, *R. alnifolia*, *Lepargyrea canadensis* and *Eleagnus commutatus* respectively, and slight morphological differences were observed between them. They were described as varieties of *Puccinia coronata* Corda and not as separate species.

Little work has been done in England on the aecidial hosts of *P. coronata*. Plowright (1889) obtained aecidia on *Rhamnus Frangula* by inoculation with teleutospores on *Dactylis glomerata* and *Festuca sylvatica*

but failed to infect this host with teleutospores on *Lolium perenne*. Hanes (1936) inoculated oats, wheat, barley, rye, *Lolium perenne*, and *L. italicum* with aecidiospores collected in the field on *Rhamnus Frangula* and *R. cathartica*. Only hypersensitive flecks were produced, although both oats and *Lolium perenne* were sometimes heavily infected in the neighbourhood in which the experiments were carried out.

Mr T. G. Jennings (unpublished work carried out at the Cambridge Botany School in 1931–2) inoculated *Rhamnus Frangula* and *R. cathartica* with teleutospores on *Calamagrostis lanceolata*, *Lolium perenne* and oats. The spores on *Calamagrostis lanceolata* infected only *Rhamnus Frangula* and those on oats and *Lolium perenne* only *Rhamnus cathartica*. He also inoculated a number of grasses with aecidiospores collected in the field on the two species of *Rhamnus*, and found that oats, *Dactylis glomerata*, *Holcus lanatus*, *Alopecurus pratensis* and *Lolium perenne* were infected only by aecidiospores from *Rhamnus cathartica*, while *Calamagrostis lanceolata* and *Phalaris arundinacea* were infected by aecidiospores from both species. These results appeared to be contradictory, since the experiments with teleutospores indicated that the varieties of *Puccinia coronata* could be clearly divided into two groups according to their aecidial host, in agreement with the results of Klebahn and Eriksson, while the experiments with aecidiospores seemed to show that they could not be differentiated in this way, in agreement with the results of Treboux and Melhus *et al.*

The experiments described in the present paper were carried out on the same lines as those of Jennings. Seedling plants of *Rhamnus Frangula* and *R. cathartica* were inoculated with sporidia from germinating teleutospores on a number of grasses, and the grasses were in turn inoculated with aecidiospores collected in the field on the two species of *Rhamnus*.

Inoculations with sporidia

(*a*) *Source of host plants*

Seedlings of *Rhamnus Frangula*, 1–2 years old, were collected in the autumn at Wicken Fen, near Cambridge, and kept in a cold frame until required for experiment in the following spring. Seedlings of *R. cathartica* were grown from seed in a greenhouse, as they were difficult to find in the field. In the few experiments in which the Canadian species, *R. alnifolia*, was used, young plants were collected in a wood near the Dominion Rust Research Laboratory, Winnipeg, and were transferred to pots in a greenhouse. Cut shoots of this species were also employed as they survived long enough in water for the production of aecidia.

(*b*) *Source of teleutospores*

Teleutospores were produced in the greenhouse at Cambridge by the varieties *calamagrostidis*, *alopecuri*, *arrhenatheri*, *avenae*, *lolii* and *holci*. They were formed mainly in the summer and early autumn and, as they would not germinate without being frozen, the plants bearing them were placed out of doors during the winter. In this way a good supply of germinating spores was obtained in the following spring.

An attempt was made to germinate teleutospores formed on oats without previous overwintering, using the technique devised by Johnson (1931) in experiments with the teleutospores of *Puccinia graminis tritici*. Pieces of straw bearing the spores were frozen in blocks of ice at $-5°$ C. for a month. They were then thawed, soaked in tap water for 24 hr., placed on filter paper in a Petri dish and subjected to alternate periods of wetting and drying, the moist and dry periods each consisting of about 24 hr. In four out of six samples slight germination was obtained, but only in one was it sufficient to cause infection of plants of *Rhamnus cathartica*. The time elapsing between the placing of the teleutospores in the refrigerator and the appearance of aecidia on the *Rhamnus* was 71 days, a period considerably shorter than would have elapsed under natural conditions between the formation of teleutospores and the production of aecidia.

Teleutospores were collected in the field on *Calamagrostis lanceolata*, *Phalaris arundinacea*, *Arrhenatherum avenaceum*, *Avena sativa*, *A. strigosa*, *Holcus lanatus* and *Lolium perenne*. They were allowed to overwinter out of doors and germinated well in the following spring.

(*c*) *Method of inoculation*

Grass leaves bearing germinable teleutospores were soaked in tap water for several hours and then tied among the young leaves of the plant to be inoculated. Both plant and teleutospore material were sprayed thoroughly with tap water from an atomizer, and placed under a bell-jar lined with damp blotting paper for 48 hr. Care was taken to keep the blotting paper moist throughout this period and the plants were sprayed at intervals to maintain a film of moisture on the surface of the leaves.

In some experiments, the plant to be inoculated was covered by a glass lamp chimney and the teleutospore material was placed on damp filter-paper in a watch-glass or Petri dish inverted over the top of this chimney. The sporidia were thus shot off their sterigmata on to the leaves of the plant. Spraying and incubation were carried out as before. This method, used by Newton & Johnson (1932) for the inoculation of barberry plants with sporidia of *Puccinia graminis*, was useful for producing scattered infection spots suitable for hybridization experiments, but, for testing the susceptibility of a *Rhamnus* species to an individual variety of rust, the first method proved more satisfactory.

After inoculation, the plants were placed out of doors enclosed in cages to exclude insects. These cages consisted of cubical wooden frames the sides of which were covered with two thicknesses of fine butter muslin, and the tops with a sheet of glass. They were effective in keeping out the majority of insects, though occasional specimens of thrips and aphides were found inside them. Each cage held nine 5½ in. pots, and only plants inoculated with the same variety of rust were placed together in a cage.

In each experiment, one plant of the *Rhamnus* species expected to be susceptible to the rust variety used was inoculated at the same time as two or three plants of the

species expected to be resistant. This was done to ensure that there was adequate opportunity for the supposedly resistant species to become infected. Experiments with an individual variety were repeated until definite conclusions could be drawn as to its power of infecting the *Rhamnus* species.

The experiments were carried out in May and June of the years 1933, 1934 and 1935.

(*d*) *Results.* The first sign of infection on the inoculated plants was the appearance of minute, yellow, slightly raised spots on the young leaves. These infection spots or pustules appeared 5–10 days after inoculation and gradually increased in size, frequently becoming confluent where they lay close together. About 3 days after their appearance, spermogonia could be discerned in them which soon began to secrete nectar.

According to Allen (1932) *Puccinia coronata* is heterothallic. A monosporidial infection will not produce aecidia unless it is sufficiently near an infection of opposite strain for the two mycelia to coalesce, or unless it is fertilized by spermatia of opposite strain. Since insects were excluded from the experimental plants, nectar from one pustule was conveyed to another on the point of a sterilized needle, each pustule receiving nectar from several others in order to ensure that the right strain was present for fertilization. When the pustules were close together and showed some coalescence, aecidia were produced without this mixing of the nectar.

Aecidia began to appear 6–10 days after fertilization. They developed on the under surface of the pustules which, by this time, had increased considerably in size, and were associated with a thickening of the lamina owing to local hypertrophy. When infection was particularly heavy aecidia were also formed on the young twigs, which became much swollen and distorted.

Table I shows the results obtained when *Rhamnus Frangula* and *R. cathartica* were inoculated with teleutospores of the different varieties of *Puccinia coronata*. These varieties can be divided into two groups according to their aecidial host: var. *calamagrostidis* produces aecidia only on *Rhamnus Frangula* while vars. *alopecuri*, *holci*, *arrhenatheri*, *lolii*, *festucae* and *avenae* produce aecidia only on *R. cathartica*.

The percentage of infection obtained with the varieties *lolii*, *festucae*, and *avenae* was rather low, chiefly owing to poor germination of the teleutospores. Infections occurred, however, always on *R. cathartica* and never on *R. Frangula*, although numerous plants of the latter were inoculated. It was concluded that *R. cathartica* was the aecidial host of these varieties, a conclusion which was confirmed, in the case of the

var. *lolii* by the results of inoculations with aecidiospores to be described later.

Table I

Infection of Rhamnus Frangula *and* R. cathartica *with teleutospores of* Puccinia coronata *Cda*

			R. Frangula			*R. cathartica*		
Variety of *P. coronata*	Host bearing teleutospores	Origin of teleutospores	No. of plants inoculated	No. of plants bearing spermogonia and aecidia	No. of plants bearing spermogonia only	No. of plants inoculated	No. of plants bearing aecidia and spermogonia	No. of plants bearing spermogonia only
Calamagrostidis	*Calamagrostis lanceolata*	Greenhouse and field	16	9	1	39	0	0
	Phalaris arundinacea	Greenhouse and field	22	15	0	48	0	2
Alopecuri	*Alopecurus pratensis*	Greenhouse	16	0	0	8	6	1
Holci	*Holcus lanatus*	Greenhouse and field	19	0	0	10	3	3
Arrhenatheri	*Arrhenatherum avenaceum*	Greenhouse and field	12	0	0	6	2	2
Lolii	*Lolium perenne*	Greenhouse and field	53	0	0	29	2	[illegible]
Festucae	*Festuca elatior*	Field	14	0	0	7	1	1
Avenae	*Avena sativa*	Greenhouse	6	0	0	6	1	0
	Avena strigosa	Field	33	0	0	11	0	3

Only one experiment gave evidence of the infection of both species of *Rhamnus* by teleutospores from a single grass species. In this two seedlings of *R. Frangula* and four of *R. cathartica* were inoculated with teleutospores collected in the field on *Phalaris arundinacea*. There was copious production of spermogonia and aecidia on *Rhamnus Frangula* but a few spermogonia were also produced on two of the plants of *R. cathartica*. These spermogonia were small and abortive, secreting very little nectar, and there was no sign of aecidial development. By the time a heavy crop of aecidia had appeared on the leaves of *R. Frangula*, the spermogonia on *R. cathartica* were much blackened and shrivelled (Pl. XXI, fig. 1).

Microtome sections were cut of the infected leaves of both species, which were fixed 13 days after inoculation in formalin-chrome-acetic solution and stained with iron-alum and haematoxylin, or diamant fuchsin and light green. The leaves of *R. Frangula* showed abundant production of mycelium, well developed spermogonia with numerous spermatia, aecidial initials on the under-surface of the leaf and considerable hypertrophy of the host cells, which were never necrotic

(Pl. XXI, fig. 2). In *R. cathartica* (Pl. XXI, fig. 3) the mycelium was poorly developed and the spermogonia small and abortive, producing few spermatia. There was no sign of aecidial initials and little hypertrophy of the host. Some of the host cells, particularly those of the upper epidermis, were collapsed and necrotic.

Hand sections, cut at the close of the experiment, 28 days after inoculation, showed well developed aecidia on *R. Frangula* and a copious mycelium ramifying through the mesophyll, which was hypertrophied but not necrotic. On *R. cathartica* the infection still showed spermogonia only; the mycelium was less well developed than in *R. Frangula* and the infection spot was surrounded by a zone of necrotic cells stretching from the upper to the lower epidermis.

Ruttle & Fraser (1927), investigating the cytology of the uredospore stage of *Puccinia coronata*, found that on the susceptible oat variety, Banner, the mycelium developed copiously, with no necrosis of the host cells, but on the partially resistant variety Cowra only a scanty mycelium appeared, and there was considerable necrosis. Hanes (1936) also found that when aecidio- and uredospores of *P. coronata* were inoculated on to resistant hosts there was poor development of mycelium and some necrosis. The type of infection obtained on *Rhamnus cathartica* therefore resembles, in many respects, that produced by uredospores on a semi-resistant host, and it seems probable that, although normally no sign of infection is produced on *R. cathartica* by var. *calamagrostidis*, occasionally the immunity may be partially broken down and development of the fungus may proceed as far as the formation of abortive spermogonia. The spread of the fungus appears, however, to be limited by the necrosis of the surrounding host cells in the same way as the spread of the uredospore stage is limited in semi-resistant hosts.

Instances of the infection of both *R. Frangula* and *R. cathartica* by teleutospores of *Puccinia coronata* from the same species of grass have been recorded in North America by Melhus *et al.* (1922), Dietz (1926*a*) and Fraser & Ledingham (1933). These workers have found that teleutospores on oats cause heavy production of aecidia on *Rhamnus cathartica* but may, also, form a few scattered spermogonia on *R. Frangula*. Because of these indecisive results, and because of the large number of *Rhamnus* and related species which may be infected by crown rust in the United States and Canada, they consider that Klebahn's division of the rust into two species does not hold for American material.

Except for the single instance described above, the varieties of *Puccinia coronata* occurring in Great Britain appear to infect either

Rhamnus Frangula or *R. cathartica* but not both, and the results thus agree with those of the European workers Klebahn, Eriksson and Mühlethaler. Whether this difference in the aecidial host is sufficient, in the light of our present knowledge of the rust fungi, to justify the division of *Puccinia coronata* Corda into two species, will be discussed later.

A few experiments were carried out with teleutospores of *P. coronata* collected in Canada on *Calamagrostis canadensis*. These teleutospores were inoculated on to *Rhamnus Frangula*, *R. cathartica* and also *R. alnifolia*, the North American species which had previously been shown by Melhus *et al.* (1922), Dietz (1926*a*) and Fraser & Ledingham (1933) to be the aecidial host of crown rust on this grass. Aecidia were obtained only on *R. alnifolia*, thus indicating that the variety of crown rust infecting *Calamagrostis* in Canada differs from that infecting the same genus in Great Britain in its aecidial, as well as in some of its uredinial hosts (Brown, 1937).

Inoculations with aecidiospores

(*a*) *Source of aecidiospores*

The aecidiospores used in these experiments were collected in the field on *Rhamnus Frangula* and *R. cathartica* during the summers of 1933 and 1934. The collections on *R. Frangula* were made at Wicken Fen, this being the only place in the neighbourhood where infected bushes were found in any abundance. The collections on *R. cathartica* were made at Wicken Fen, at the Cambridge University Farm and in the University Botanic Garden.

(*b*) *Method of inoculation*

The aecidiospores were inoculated on to the grasses and oat varieties used in the experiments with uredospores (Brown, 1937), and listed in Tables II and III. The seedling grasses were inoculated when five or six leaves had developed, and the oats were inoculated on the first leaf when only this had emerged. The method of inoculation was as follows: the aecidiospores were scraped off a number of infected leaves into a sterilized Petri dish and were thoroughly mixed together to prevent discrepancies in the results due to the occurrence of only one variety of rust in a single aecidium or a single aecidial pustule. They were then transferred on a sterile scalpel to the upper surface of the leaves to be inoculated, which were marked with Indian ink for identification. The plants were then sprayed thoroughly with tap water by means of an atomizer and were incubated under bell jars standing over water for 48 hr. After the incubation period, they were placed on the greenhouse bench and covered with cellophane cages. As a rule, each collection of aecidiospores was inoculated on to the complete range of grasses and oat varieties, and the inoculations with spores from the two species of *Rhamnus* were always made on the same day, so that direct comparisons could be made of their infective capacities.

Uredospore pustules began to appear about 10 days after inoculation, and when these were fully developed, about 5 days later, notes were taken of the number of plants infected and the type of infection produced. The infection types were classified according to the scheme drawn up by Stakman & Levine (1922) for the infection types produced on wheat by *Puccinia graminis tritici*, and were divided into five classes, designated by the symbols 0 to 4, as described below:

Table II

Results obtained on inoculation of grasses with aecidiospores collected in the field on Rhamnus Frangula *and* R. cathartica. *Summers* 1933 *and* 1934

Species inoculated	Aecidia on *R. Frangula*: Total no. of plants: Inoculated	Infected	Infection type	Aecidia on *R. cathartica*: Total no. of plants: Inoculated	Infected	Infection type	
Lolium perenne	34	0	0	78	43	0, 3, 4	Group I
Holcus lanatus	34	0	0	69	31	0, 3, 4	
Alopecurus pratensis	27	0	0	64	23	0, 2, 3, 4	
Arrhenatherum avenaceum	34	0	0	58	26	0, 4	
Festuca elatior	30	0	0	48	16	0, 3, 4	
Dactylis glomerata	34	1	0, 2	65	7	0, 1, 2, 3	Group II
Calamagrostis lanceolata	44	42	4	55	25	0, 1, 2, 3, 4	
Phalaris arundinacea	34	32	3, 4	63	2	0, 1, 2	
Agropyron repens	30	0	0	45	0	0	Group III
Bromus sterilis	29	0	0	54	0	0	
Agrostis palustris	16	0	0	34	0	0	

Host reaction	Symbol	Infection type
Highly resistant	0	No uredospore pustules developed; necrotic or chlorotic flecks usually present.
Very resistant	1	Pustules minute and isolated; usually accompanied by pronounced necrosis; necrotic spots often produced without development of pustules.
Moderately resistant	2	Pustules small to medium in size; surrounded by necrotic or markedly chlorotic areas; necrotic spots rarely without pustules.
Moderately susceptible	3	Pustules fairly abundant; medium sized; no necrosis, but chlorosis usually present.
Very susceptible	4	Pustules large and abundant; often confluent and showing copious production of uredospores; no necrosis but occasional slight chlorosis.

(The *X* type of infection in which all five types are found on the same leaf did not occur in these experiments.)

The experiments were carried out in June and July 1933 and 1934 in an unheated greenhouse whose average daily temperature ranged from 58 to 79° F.

(c) *Results*

(i) *Inoculation of grass species.* The results obtained on the inoculation of the grass species are shown in Table II. It was found that individual plants of the same species differed very much in their degree of infection

even when inoculated with aecidiospores from a single sample. This was probably due partly to the occurrence of a number of different varieties of rust in the aecidial inoculum and partly to variations in the constitution of the host plants.

In spite of these variations, the grasses could be clearly divided into three groups:

I. Grasses which were infected by aecidiospores from *Rhamnus cathartica*, but not by those from *R. Frangula*.

II. Grasses which were infected by aecidiospores from both species of *Rhamnus*.

III. Grasses which were not infected by aecidiospores from either species.

The grasses falling into Group I are *Lolium perenne*, *Holcus lanatus*, *Alopecurus pratensis*, *Arrhenatherum avenaceum* and *Festuca elatior*. They showed infections of the 3 and 4 types on 33–55% of the plants inoculated with aecidiospores from *Rhamnus cathartica*, but only flecking or no sign of infection on the plants inoculated with aecidiospores from *R. Frangula*.

In order to identify the varieties of rust which caused the infections, uredospores from each grass were inoculated on to as many as possible of the series of grass species used for the differentiation of varieties in the experiments with uredospores (Brown, 1937). It was shown that the infections on *Lolium perenne* and *Festuca elatior* were due to the variety *lolii*, those on *Holcus lanatus* to the variety *holci*, those on *Arrhenatherum avenaceum* to the variety *arrhenatheri*, and those on *Alopecurus pratensis* to the two varieties *alopecuri* and *lolii*. This last result is in agreement with the findings in the experiments with uredospores, in which the variety *lolii* was not confined to *Lolium perenne* but was capable of causing moderate infection on certain other grasses, including *Alopecurus pratensis*. The variety *festucae* did not appear to be present in this aecidial inoculum although it was observed in the neighbourhood in the uredospore stage.

The aecidiospores obtained from *Rhamnus cathartica* thus belonged to the varieties *lolii*, *holci*, *arrhenatheri*, and *alopecuri*. This is in agreement with the results of the experiments with teleutospores, in which the sporidia of these varieties infected *R. cathartica* but not *R. Frangula*.

The grasses of Group II, which were infected by aecidospores from both species of *Rhamnus*, were *Calamagrostis lanceolata*, *Phalaris arundinacea* and *Dactylis glomerata*. The first two were infected more heavily

by the aecidiospores from *Rhamnus Frangula* and the last by those from *R. cathartica*.

In the experiments with teleutospores; rust on *Calamagrostis lanceolata* and *Phalaris arundinacea* gave rise to aecidia only on *Rhamnus Frangula* and did not infect *R. cathartica*, so that the results of the two sets of experiments seemed to be contradictory. The apparent discrepancy, however, was explained when the varieties responsible for the infections were identified as far as was possible with the limited amount of inoculum available.

The heavy infections obtained on *Calamagrostis lanceolata* and *Phalaris arundinacea* when inoculated with aecidiospores from *Rhamnus Frangula* were shown to belong to the variety *calamagrostidis*, but the infections produced on these grasses by aecidiospores from *R. cathartica* were so scanty that it was impossible to inoculate more than a small number of the differential hosts. A few tests were, however, carried out with uredospores from *Calamagrostis lanceolata*, and the results are shown in Table III. In the circumstances it was necessary in planning the tests, to decide which varieties of rust were most likely to be present, and to choose for inoculation those grasses which would differentiate them most clearly. In the experiments with uredospores (Brown, 1937) it was found that *Calamagrostis lanceolata* was infected most heavily by var. *calamagrostidis*, but was also somewhat susceptible to vars. *lolii*, *alopecuri*, *festucae* and *avenae*. Vars. *lolii* and *alopecuri* were known to be present in the aecidial inoculum so that it seemed likely that they, or var. *calamagrostidis*, were responsible for the infection. Of these three varieties: var. *alopecuri* causes heavy infection on *Alopecurus pratensis*, light infection on *Calamagrostis lanceolata* and no infection on *Lolium perenne*; var. *lolii* infects *Lolium perenne* heavily and *Alopecurus* and *Calamagrostis* with infection types ranging from 1 to 3; while var. *calamagrostidis* only infects *Calamagrostis*. The varieties could therefore be differentiated by means of these three grasses, which were accordingly used in the experiments. Sufficient inoculum was not however available for each of them to be used in every test.

Table III shows that in tests I and II *Lolium perenne* was heavily infected and the variety present was therefore var. *lolii*; moreover, the lack of infection on *Calamagrostis lanceolata* confirmed the absence of var. *calamagrostidis*. In test III, only *Lolium perenne* was inoculated, and as it was not infected, it was impossible to draw any conclusions as to the nature of the variety, except that it was probably not var. *lolii*. In test IV, *Alopecurus pratensis* was infected but not *Lolium perenne*, so

that the infection was evidently due to var. *alopecuri*. In test V only *Calamagrostis lanceolata* was infected, but the pustules were very small and some were surrounded with necrotic areas, which are not obtained when the grass is infected with var. *calamagrostidis*. The spore production was so scanty that it was impossible to maintain the rust for further experiment, and no conclusions could be reached as to the variety responsible for the infection.

Table III

Infection types produced by first generation of uredospores on Calamagrostis lanceolata *inoculated with aecidiospores from* Rhamnus cathartica. (*Tests carried out June-July* 1934)

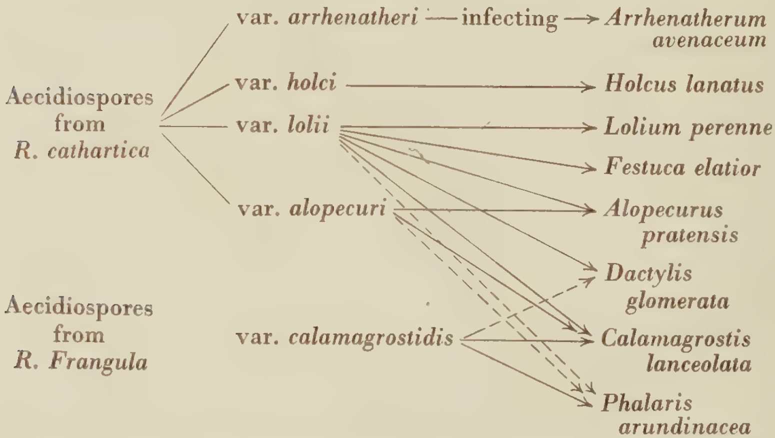
Test no.	Source of aecidiospores	Infection type produced on *C. lanceolata*	Grasses inoculated with uredospores from *C. lanceolata*	No. of plants Inoculated	No. of plants Infected	Infection type	Variety causing infection
I	*R. cathartica* University Farm	2	*Lolium perenne*	2	2	4	*lolii*
II	*R. cathartica* University Farm	2	*Lolium perenne*	1	1	4	*lolii*
			Calamagrostis lanceolata	1	0	0	
III	*R. cathartica* Botanic Garden	3	*Lolium perenne*	2	0	0	?
IV	*R. cathartica* Botanic Garden	4	*Lolium perenne*	2	0	0	*alopecuri*
			Alopecurus pratensis	2	2	4	
V	*R. cathartica* Wicken Fen	3	*Lolium perenne*	1	0	0	?
			Alopecurus pratensis	1	0	0	
			Calamagrostis lanceolata	1	1	2 to 3	

Some of the infections obtained on *Calamagrostis lanceolata* when inoculated with aecidiospores from *Rhamnus cathartica* are due evidently to the vars. *lolii* and *alopecuri*. These varieties are also capable of producing small uredopustules on *Phalaris arundinacea* and it seemed likely that they might have caused the slight infections obtained on this grass.

In the case of *Dactylis glomerata*, the uredospores produced as a result of inoculation with aecidiospores from *Rhamnus cathartica* were found to belong to var. *lolii*, which had been shown in the experiments with uredospores to cause heavy infection on this grass. The single plant infected by aecidiospores from *R. Frangula* did not yield sufficient spores for further inoculation to be carried out, so that it was impossible to identify the variety present. The inoculum from *R. Frangula* appeared to contain only var. *calamagrostidis* and, as this variety had been shown occasionally to produce pustules of types 1 and 2 on *Dactylis glomerata*, it was thought that it might have caused the infection in this instance.

It seems, therefore, that although the grasses in Group II are infected by aecidiospores from both *Rhamnus Frangula* and *R. cathartica*, the varieties causing the infections are different in the two cases. Text-fig. 1 indicates diagrammatically the varieties found on the two alternate hosts, and the way in which they are considered to infect the grasses of Groups I and II.

In the light of this interpretation, the results of the experiments with aecidiospores are no longer opposed to those of the experiments with teleutospores, since they indicate that vars. *holci*, *arrhenatheri*, *lolii* and



Text-fig. 1. Diagram showing the constitution of the aecidiospore collections made on *Rhamnus cathartica* and *R. Frangula* and the grasses which they infected. ⟶ Variety identified by experiment. - - -→ Identity of variety inferred from evidence obtained in related experiments.

alopecuri have their aecidial stage on *R. cathartica*, and var. *calamagrostidis* has its aecidial stage on *R. Frangula*. They are, also, in accordance with the results of Klebahn, Eriksson and Mühlethaler, in so far as they show that the varieties of *Puccinia coronata* are markedly specialized in relation to their aecidial as well as to their uredinial hosts.

It is possible, moreover, that some of the results obtained by Treboux (1912, 1914), which appeared to be so much at variance with those of other European workers, may be explained in the same way as the infection of *Calamagrostis lanceolata* with aecidiospores from both species of *Rhamnus* has been explained in the present experiments. For example, Treboux (1912) found that *Phalaris arundinacea* was infected to some extent by aecidiospores from *Rhamnus cathartica*, although Klebahn

(1895, 1896, 1898) and Eriksson (1897, 1909) had found that rust from this grass had its aecidial stage only on *R. Frangula*. Amongst the other hosts which Treboux infected with the spores from *R. cathartica*, were *Avena sativa*, *Alopecurus pratensis* and *Lolium perenne*, and it is thus possible that his infections on the *Phalaris* were due to the varieties *avenae*, *alopecuri* or *lolii*, all of which have been shown to infect this grass.

The grasses occurring in Group III were *Agropyron repens*, *Bromus sterilis* and *Agrostis palustris*. They bore no pustules when inoculated with aecidiospores from either species of *Rhamnus*, although their inoculated leaves were usually flecked.

During the years in which the experiments were carried out, no crown rust was found in the neighbourhood of Cambridge on either *Agropyron* spp. or *Bromus* spp., so that the varieties corresponding to the f.sp. *agropyri* recorded by Eriksson (1897, 1909) in Hungary and Moravia and the f.sp. *bromi* recorded by Mühlethaler (1911) in Switzerland appear to be absent from Cambridgeshire. Slight infection of *Agrostis palustris* was observed in the field, but it proved impossible to cultivate the rust from this grass in the greenhouse. The variety responsible for the infection is, therefore, unknown.

(ii) *Inoculation of oat varieties.* The results of the inoculation of a number of oat varieties with aecidiospores from both species of *Rhamnus* are shown in Table IV. The plants were only slightly infected, whether

Table IV

Results obtained on inoculations of oat varieties with aecidiospores collected in the field on Rhamnus Frangula *and* R. cathartica. *Summers* 1933 *and* 1934

Variety inoculated	Aecidiospores on *R. Frangula*: Total no. of plants Inoculated	Infected	Infection type	Aecidiospores on *R. cathartica*: Total no. of plants Inoculated	Infected	Infection type
Avena sativa:						
Abundance	18	0	0	36	0	0
Black Tartary	18	0	0	26	0	0
Fyris	15	0	0	23	3	0, 1, 2 –
Grey Winter	16	0	0	21	1	0, 1
Hede	12	0	0	20	1	0, 1
Mesdag	12	0	0	19	1	0, 1
Scotch Potato Oat	5	0	0	9	0	0
Thousand Dollar	10	0	0	17	0	0
White Cross	12	3	0, 1	24	0	0
Avena strigosa:						
Cc 2596	18	0	0	27	2	0, 1, 2

they were inoculated with aecidiospores from *R. Frangula* or *R. cathartica* and this was somewhat surprising, as oats are frequently heavily infected by *Puccinia coronata* in this country. It appeared likely that the poor infection was due to the absence of the appropriate variety of rust in the aecidial inoculum. This was confirmed in the two cases in which sufficient uredospores were obtained for the variety causing the infection to be identified. Spores produced on Fyris by inoculation with aecidiospores from *Rhamnus cathartica* were found to belong to the variety *alopecuri*, and those produced on White Cross inoculated with aecidiospores from *R. Frangula* to belong to the variety *calamagrostidis*. Both these varieties had been shown in the experiments with uredospores to be capable of causing slight infection on oats. It appeared, therefore, that the variety *avenae* was absent from the aecidial inoculum obtained in the neighbourhood of Cambridge in 1933 and 1934 and this was confirmed by the fact that the oat crops were quite free from crown rust in those years.

Experiments on the effect of passage through the aecidial host upon the pathogenicity of varieties of *Puccinia coronata*

Dietz (1926*b*) found that the pathogenicity of certain varieties of *Puccinia coronata* was markedly altered by their passage through the aecidial host. Uredospores of var. *avenae*, for example, infected *Holcus lanatus* only slightly and *Calamagrostis canadensis* not at all, but aecidiospores of the same variety infected both grasses very heavily. Dietz could give no explanation of this apparent effect of the alternate host upon the pathogenicity of the variety but the genetical work resulting from Craigie's discovery of heterothallism in the rusts has thrown light upon this matter. Newton *et al.* (1930 *a*, *b*; 1932) have shown that the physiologic races of *Puccinia graminis tritici* are usually heterozygous for pathogenicity, and that when a race is passed through the alternate host, the segregation and recombination of the factors governing this character generally lead to the production of a number of different races, none of which may be identical with the parent. Moreover, it has been shown by Waterhouse (1929) and Newton *et al.* (1930 *a*, *b*, 1932) that when two physiologic races are hybridized on the barberry, races differing from either of the parents may be found in the aecidial progeny. Similar results have been obtained by Johnson *et al.* (1932, 1933) and Stakman *et al.* (1930, 1934) in crosses between varieties of *P. graminis*, although there is a greater degree of inter-sterility between the varieties than between the physiologic races.

It seems possible that the changes in pathogenicity obtained by Dietz in *P. coronata* were due either to the heterozygous nature of the variety used, or to accidental hybridization. In the present investigation, some experiments were carried out to determine the effect of self-fertilization and hybridization on the varieties of *P. coronata* occurring in this country.

(*a*) *Experiments on self-fertilization.* Plants of *Rhamnus Frangula* and *R. cathartica* were inoculated with sporidia from germinating teleutospores as previously described, and were placed out of doors in insect-proof cages. When the spermogonia were well developed, the "selfing" of the variety was carried out by transferring nectar from one infection pustule to another on a sterilized needle. Nectar from as many pustules as possible was intermixed so that the + and − strains had ample opportunity of coming into contact. Where the infection pustules were numerous and confluent, this mixing of the nectar was unnecessary.

In order to investigate the pathogenicity of the aecidial progeny, the aecidiospores were inoculated on to as many as possible of the series of grasses used for the differentiation of varieties in the experiments with uredospores (Brown, 1937). The inoculations were carried out with a mixture of spores from as many aecidia as were available, instead of with spores from a single aecidial cup, as in the experiments of Newton & Johnson (1932). This was done because no grass or cereal was available which was susceptible to all the varieties of the rust, and it was necessary to inoculate as many grasses as possible so as to afford any varieties or races which might arise an opportunity of infecting a congenial host.

The varieties used were *calamagrostidis*, *holci* and *alopecuri*. Aecidia of var. *calamagrostidis* were produced on *Rhamnus Frangula* by inoculation with teleutospores formed in the greenhouse on *Calamagrostis lanceolata* and *Phalaris arundinacea*; and aecidia of vars. *holci* and *alopecuri* were produced on *Rhamnus cathartica* by means of teleutospores formed in the greenhouse on *Holcus lanatus* and *Alopecurus pratensis*, respectively. Table V shows the infection types given by the aecidiospores of these three varieties compared with those given on the same grasses by their uredospores as described previously (Brown, 1937).

Close agreement is shown between the infection types given by the aecidiospores and the uredospores and, where small differences do occur, e.g. in var. *alopecuri* on *Phalaris arundinacea* and *Arrhenatherum avenaceum*, they are too small to be significant, especially when the genetic impurity of the host plants is taken into consideration. It appears, therefore, that the pathogenicity of these varieties is not appreciably altered by their passage through the aecidial host.

Table V

Infection types given by aecidiospores of vars. calamagrostidis, holci *and* alopecuri *compared with those given by their uredospores*

Grass species inoculated	var. *calamagrostidis* Aecidio-spores	var. *calamagrostidis* Uredo-spores	var. *holci* Aecidio-spores	var. *holci* Uredo-spores	var. *alopecuri* Aecidio-spores	var. *alopecuri* Uredo-spores
Calamagrostis lanceolata	4	3, 4	0	0	0, 2, 3	0, 1, 2, 3
Phalaris arundinacea	3, 4	3, 4	—	0	0, 1, 3	0, 1, 2
Dactylis glomerata	0	0, 1	—	0	0	0, 1
Alopecurus pratensis	0	0	0	0	4	4
Lolium perenne	0	0	0	0	0	0
Holcus lanatus	0	0	4	4	0	0
Arrhenatherum avenaceum	0	0	0	0	0, 1	0
Bromus sterilis	0	0	—	0	0	0
Avena sativa (var. Abundance)	—	0	—	0	0	0, 1
No. of experiments carried out with aecidiospores of each variety	4		2		4	

Table VI

Infection types produced by aecidiospores on Rhamnus Frangula *and* R. cathartica *infected by teleutospores collected in the field*

Grass species inoculated	Source of teleutospores: *Calamagrostis lanceolata*	*Phalaris arundinacea*	*Holcus lanatus*	*Arrhenatherum avenaceum*
Calamagrostis lanceolata	3, 4	3, 4	0	—
Phalaris arundinacea	3	3, 4	—	0
Dactylis glomerata	0, 2	0, 2	—	—
Alopecurus pratensis	0	0	0	—
Lolium perenne	0	0	0	0
Holcus lanatus	0	0	4	0
Arrhenatherum avenaceum	0	0	0	4
No. of experiments carried out with aecidiospores from each source	4	5	2	2

Experiments were carried out with the aecidiospores produced on the two *Rhamnus* species when inoculated with teleutospores collected in the field on *Calamagrostis lanceolata*, *Phalaris arundinacea*, *Holcus lanatus* and *Arrhenatherum avenaceum*, and the results are shown in Table VI. Although, in some cases the aecidial material was very scanty, the infection types produced agree with those given by the uredospore cultures of the varieties appropriate to the grasses bearing the teleutospores. Aecidiospores produced by inoculation with teleutospores on *Calamagrostis lanceolata* and *Phalaris arundinacea*, give infection types

similar to those of uredospore cultures of var. *calamagrostidis*; and aecidiospores produced by inoculation with teleutospores on *Holcus lanatus* and *Arrhenatherum avenaceum* give infection types similar to those of uredospore cultures of vars. *holci* and *arrhenatheri* respectively. These experiments indicate that the varieties of *Puccinia coronata* are not altered appreciably by their passage through the aecidial host.

(*b*) *Experiments on the hybridization of varieties.* The technique employed in these experiments was essentially the same as that used by Newton & Johnson (1932) for the hybridization of physiologic races of *Puccinia graminis tritici*. Isolated spermogonial pustules were chosen and nectar from the pustules of one variety was transferred to those of another on the point of a sterilized needle. Each pustule received nectar from several pustules of the variety with which it was being crossed and the needle was sterilized between each transfer. Whenever possible reciprocal crosses were made and in each experiment some of the pustules were "selfed", i.e. nectar was transferred to them from other pustules of the same variety. Crosses were made between the following varieties: *calamagrostidis* × *holci*, *calamagrostidis* × *alopecuri*, *holci* × *alopecuri* and *holci* × *lolii*. Out of ten crosses made, aecidia were obtained only in four and, in these four, only on seven out of the nineteen "crossed" pustules. On the "selfed" pustules aecidia were obtained in every case except one. The infection types produced on certain grasses by the aecidiospores from the "crossed" pustules agreed so closely with those produced by aecidiospores from the "selfed" that it seems likely that the former were not of hybrid origin, but arose through accidental selfing. These varieties of *Puccinia coronata* do not appear to hybridize readily and resemble in this respect the varieties of *P. graminis* investigated by Johnson *et al.* (1932) and Johnson & Newton (1933).

Discussion

The results obtained show that the varieties of *Puccinia coronata* occurring in Great Britain are markedly specialized in relation to their alternate hosts. The varieties *alopecuri*, *avenae*, *arrhenatheri*, *festucae*, *holci* and *lolii* have their aecidial stage on *Rhamnus cathartica* and do not infect *R. Frangula* and the variety *calamagrostidis* has its aecidial stage on *R. Frangula* and only occasionally infects *R. cathartica*, with the production of abortive spermogonia.

Klebahn (1892) used this difference in aecidial host relationship as a basis for dividing the original species, *Puccinia coronata* Corda, into two; *P. coronata* Kleb. and *P. coronifera* Kleb., but, in the light of our present

knowledge of specialization of parasitism in the rust fungi, it is doubtful if such a difference should be used as a specific criterion. The definition of a species employed by Arthur in his *Manual of the Rusts in United States and Canada* (Purdue Research Foundation, 1934) is that formulated by the American Phytopathological Society in 1925 (*Phytopathology*, **15**, 316). It appears to be generally accepted by mycologists and is as follows: (The term species shall be applied to) "a group of individuals which can be segregated on the basis of morphologic characters of such a nature as to be applicable and determinable by mycologists and pathologists in general and such as will be available for general, taxonomic purposes." Cultural characters and differences in host relationship are not used as criteria for species differentiation, and as there are no morphological characters whereby the varieties of crown rust infecting *Rhamnus cathartica* can be distinguished from those infecting *R. Frangula*, there seems to be no reason why they should be considered to belong to different species. The problem is merely one of physiologic specialization involving the aecidial as well as the uredinial stage and, if a difference in the aecidial host were used as a specific distinction, the varieties of crown rust which occur in Canada and the United States and have their aecidia on *Rhamnus alnifolia*, *Lepargyrea canadensis* and *Eleagnus commutatus* should all be considered as separate species.

A practical difficulty in dividing crown rust into two species is the impossibility of assigning a collection of rust made in the field either to *Puccinia coronata* Kleb. or to *P. coronifera* Kleb. (*P. Lolii* Niels.) without carrying out cultural experiments. It is not sufficient to take the uredinial host on which the rust is found as an indication of the species to which it belongs, because the present experiments have shown that a "*coronifera*" variety of rust may cause quite heavy infection on a "*coronata*" host. For example, var. *lolii* may cause an infection of type 3 upon *Calamagrostis lanceolata*, so that if rust is found on this grass in the field, it is impossible to tell without experiment whether it belongs to var. *calamagrostidis*, which has its aecidial stage on *Rhamnus Frangula*, or to var. *lolii*, which has its aecidial stage on *R. cathartica*. It would seem to be in the interests both of accuracy and of convenience if the use of the specific names *Puccinia coronata* Kleb. and *P. coronifera* Kleb. (*P. Lolii* Niels.) were discontinued and the original name *P. coronata* Corda were employed to designate all those types of rust which have in common the coronate teleutospores and other morphological characters which we associate with crown rust.

Summary

1. Experiments were carried out to determine the relationship between the varieties of *Puccinia coronata* Corda previously isolated in Great Britain and the two species of *Rhamnus*, *R. Frangula* and *R. cathartica*, which are the alternate hosts of the rust in this country.

2. The experiments included: (*a*) the inoculation of seedling plants of the *Rhamnus* species with sporidia from germinating teleutospores of the different varieties; and (*b*) the inoculation of a number of grasses with aecidiospores collected in the field on the two species of *Rhamnus*.

3. The varieties were found to show a considerable degree of specialization in their relation to the alternate hosts, vars. *alopecuri*, *arrhenatheri*, *avenae*, *festucae*, *holci* and *lolii* producing aecidia on *R. cathartica* only and var. *calamagrostidis* on *R. Frangula* only.

4. The difference in aecidial host relationship of the varieties is not considered to be an adequate criterion for species differentiation, and it is suggested that the use of the names *Puccinia coronata* Kleb. and *P. coronifera* Kleb. (*P. Lolii* Niels.) be discontinued and the rust be designated by the original name *P. coronata* Corda.

5. The pathogenicity of the varieties was not altered appreciably by passage through the alternate host and the varieties did not appear to hybridize readily.

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Fig. 1.

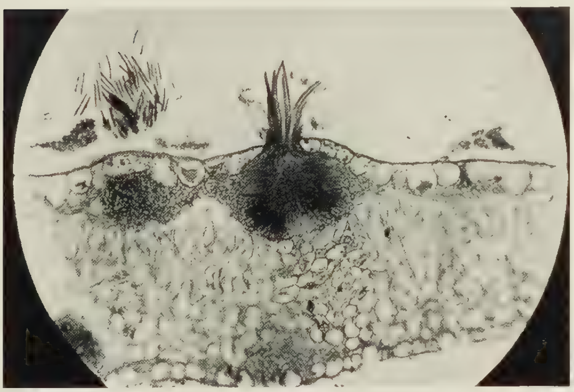

Fig. 2.

Fig. 3.

BROWN.—A STUDY OF CROWN RUST, *PUCCINIA CORONATA* CORDA, IN GREAT BRITAIN (pp. 506–527)

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EXPLANATION OF PLATE XXI

Fig. 1. Leaves of *Rhamnus Frangula* and *R. cathartica* inoculated 30. v. 34 with teleutospores on *Phalaris arundinacea*. Photograph taken 21. vi. 34. Natural size. Leaf (*a*) *Rhamnus Frangula*, upper surface showing numerous infection pustules. Leaf (*b*) *R. Frangula*, lower surface showing aecidia. Leaf (*c*) *R. cathartica*, upper surface showing shrivelled infection pustules with abortive spermogonia. Leaf (*d*) *R. cathartica*, lower surface showing shrivelled infection pustules with abortive spermogonia.

Fig. 2. Transverse section of leaf of *Rhamnus Frangula* inoculated with teleutospores on *Phalaris arundinacea*, showing normal spermogonium and absence of necrosis in host cells. × 170.

Fig. 3. Transverse section of leaf of *Rhamnus cathartica* inoculated with teleutospores on *Phalaris arundinacea*, showing abortive spermogonium and necrosis of host cells. × 170.

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SOME SPECIES OF *PYTHIUM* PARASITIC ON WHEAT IN CANADA AND ENGLAND[1]

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(With Plate XXII and 2 Text-figures)

The large number of publications dealing with *Pythium* parasites on the roots of graminaceous hosts in widely separated parts of the world during the last decade may be taken as evidence of the economic importance of this group of organisms. The crops most particularly affected are wheat in Canada, Italy, India, and Japan; maize in the United States, the Philippines, Hawaii, and Italy; sugar-cane in the U.S.A., Hawaii, the Philippines, Mauritius, and India; rice in Java, Japan, Portugal, and the U.S.A.; grasses and turf in the U.S.A. and Holland, as well as in many other countries. *Pythium* root rots or seedling blights have been reported from time to time on oats from Britain, Canada, Denmark, the U.S.A., and Holland; on barley from Tunis, Canada, and Japan; and on milo (*Sorghum* sp.) from the U.S.A. Earlier literature reviews on the subject, together with the investigations of the root rot on cereals, are to be found in previous papers by Vanterpool *et al.* (1930, 1932, 1935).

The author studied certain *Pythium* parasites of wheat in England during the greater part of the winter months of 1935–6. The species obtained were compared with species ordinarily considered as the primary parasitic agents in browning root rot of wheat on the Canadian prairies. The work was completed after the author's return to Canada. The combined results are embodied in this paper.

Pythium damage has attracted little attention until comparatively recently. In so far as the root rot of cereals is concerned, diseased plants resemble, and have doubtless been mistaken for, plants suffering from drought, from excess salts or alkali, or from nutrient deficiencies, and in the case of winter cereals, from prolonged wet, cold, dull weather. Indeed, there is evidence that with continuous cropping or improper crop

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rotation and farm practices, nutrient deficiencies or unbalanced nutrition do predispose the host plants to attack by the parasite (Carpenter, 1934; Cooke, 1934; Vanterpool, 1935). Difficulty, too, is commonly experienced in isolating *Pythium* parasites of roots when these are known definitely to be present in diseased portions of the root. The recognized method (Rands & Dopp, 1934; Vanterpool & Truscott, 1932) for isolating phycomycetous root pathogens is likely to give best results and should be employed. A difficulty frequently confronts the investigator in this field. The rather limited cultural requirements of many species for the formation of sexual organs and for the initiation of zoospore discharge make it often difficult to obtain oogonia and antheridia, and zoospores, in culture. The specific identity of the fungus is, therefore, either not ascertained or greatly delayed.

It is perhaps significant that, with few exceptions, all the species concerned belong to the nematosporangial group of *Pythium*, the most important being *P. arrhenomanes* Drechsl., considered in its broader sense, and *P. graminicolum* Subram.

METHODS

In the investigation in England, wheat-seedling material from two sources was used for isolation purposes. In one case wheat was grown in pots of soil collected the previous summer from fields in wheat-growing districts near Slough, Cambridge, and Ramsgate. These soil samples, which were kindly given to me by Mr S. D. Garrett, had been air-dried for a considerable time. After five or six weeks the seedlings were washed free of soil and many root lesions were found to contain *Pythium* oospores. The subsequent isolation procedure was according to the method previously stated. In the second instance, wheat seedlings with root systems and adhering soil were procured from fields at Harpenden, Reading, Jealott's Hill, and Littlewood, during the last week of March 1936, and cultures obtained as before. Then followed a preliminary cultural study in which only one or two strains of each morphologically similar form from one source were kept. The pathogenicity of these isolates to wheat was then conducted in small Erlenmeyer flasks as described in a previous paper (Vanterpool & Truscott, 1932). Those strains showing parasitic ability were reserved for further cultural studies and pathogenicity tests on wheat in sterilized potted soil. Maize-meal agar, carrot maize-meal agar, and water agar containing wheat root tips were the media used in the comparative morphological study in Petri dishes, the last-named medium being particularly suitable for the development and study of

oogonia and antheridia, especially in the vicinity of the wheat root tips. All growth rates were obtained from colonies on carrot maize-meal agar. Water cultures, with and without steam-sterilized wheat root tips, were used mainly for zoospore discharge, but were also supplementary to the solid media.

Pathogenic species

(1) *Pythium arrhenomanes* Drechsl. (Drechsler, 1928, 1936; Rands & Dopp, 1934)

This species is here considered in the wider sense of Rands & Dopp (1934) to include those forms morphologically similar in the radiating aspect of the numerous diclinous antheridial stalks, regardless of whether or not lobulate sporangia have been observed. It is very widely distributed and probably causes more damage to graminaceous crops than any of the other species under consideration. It has been reported as the cause of more or less severe root rots of maize in the U.S.A. (Johann *et al.* 1928), the Philippines (Roldan, 1930, 1932), Hawaii (cf. Sideris, 1931) and Canada (unpublished information); of wheat in Canada (Vanterpool & Truscott, 1932; Vanterpool, 1935), and Hawaii (cf. Sideris, 1931); of sugar-cane in the U.S.A. (Edgerton *et al.* 1929; Rands & Dopp, 1934), Hawaii (cf. Drechsler, 1936; Rands & Dopp, 1934), the Philippines (Roldan, 1930), India (Subramaniam, 1936), and Mauritius (Shepherd, 1933), and of milo (*Sorghum* sp.) in the U.S.A. (Elliott *et al.* 1932, 1937).

Two English strains were obtained: one from wheat seedlings grown in soil, which had been air-dried for several weeks, from plots at the Rothamsted Experimental Station, and the other from seedlings collected from a clay-loam soil in a field near Littlewood, in April 1936. The Rothamsted soil was procured through the kindness of Dr J. B. Marshall, and the Lincolnshire sample through that of Mr R. V. Tipler. The Rothamsted strain was readily obtained from soil which had received ammonium sulphate fertilizer only, or ammonium sulphate plus minerals, over a number of years. Both the English strains were larger in average spore size than the Canadian type culture (Vanterpool *et al.* 1932) and failed to produce (lobulate) sporangia; they closely resembled other non-lobulate, large-spored Canadian strains which were available for study. The Rothamsted strain had a growth rate approximately equal to that of the Canadian type culture, while the Lincolnshire strain had a lower growth rate. Both the English strains proved to be highly pathogenic to Marquis wheat.

(2) *Pythium graminicolum* Subram. (Subramaniam, 1928; Drechsler, 1936)

This species was first described as a parasite of wheat in India (Subramaniam, 1928) and is frequently obtained from rotting sugar-cane roots in the U.S.A. (Rands & Dopp, 1934), Porto Rico (Drechsler, 1936), India (Subramaniam, 1936), and Hawaii (Carpenter, 1934; cf. Drechsler, 1936) where, together probably with *P. arrhenomanes*, it is one of the chief factors in the decline or failure of certain sugar-cane varieties. Confusion arising between the identity of this species, the original type culture of which is no longer available, and *P. arrhenomanes* has recently been cleared up by Drechsler (1936). The writer has not encountered any forms in Canada which he considers to belong to this species.

The English strain was isolated in December 1935 from wheat seedlings grown in field soil collected the previous summer near Cambridge. In the interval the soil had become thoroughly air dried. In spore size this strain is more in agreement with Subramaniam's plant (1928) than with Miss Matthew's (1931). It was more pathogenic to Marquis wheat than a strain from sugar-cane received from Dr C. Drechsler six years ago.

(3) *Pythium volutum* Vanterpool & Truscott (1932; Van Luijk, 1934)

This species was first reported as a parasite of wheat and oats in Canada (Vanterpool & Truscott, 1932) and later of grasses in Holland (Van Luijk, 1934). Though closely resembling its congener *P. arrhenomanes*, the writer does not consider that it falls within the ambit of the latter species as defined by Rands & Dopp (1934). Its chief characteristics are sufficiently distinct to warrant specific rank. It differs from *P. graminicolum* chiefly in having its oospores usually free within the oogonium, in producing sporangia rarely, and in being less frequently androgynous.

Two English strains, agreeing closely with the Canadian type species in general cultural characters, in antheridial disposition, and in the wrapping of antheridial branches about the oogonial stalk under certain cultural conditions, were obtained in December 1935 from wheat seedlings in potted soil; one from soil collected near Ramsgate, and the other from Slough soil. These were farm soils typical of the districts, with reactions of pH 8·0 and 6·8, respectively. The English strains are severely parasitic on wheat seedlings.

Text-fig. 1.

(4) *Pythium tardicrescens* n.sp. (Pl. XXII, figs. 1, 2 and 4; Text-fig. 1)

This form is found both in Canada and England. It was first isolated in Canada in 1929, from wheat roots affected with browning root rot and is now considered next in importance to *P. arrhenomanes* among the causal agents of this disease. Over a number of years it has been obtained from widely separated points in the wheat-growing area of Saskatchewan. It is slow-growing and exacting in its cultural requirements and is not as readily isolated as some of the other species. No growth occurs at 30° C. It is further characterized by the clumping of the oogonial contents into large, lustrous oil drops which stain red with Sudan III. Only a small percentage of oospores mature. Though not as actively pathogenic to wheat under artificial conditions as *P. arrhenomanes* and *P. aristosporum*, it produces brown root lesions strikingly similar to those obtained under field conditions (Pl. XXII, fig. 2). Oospores are found scattered sparsely through these lesions, occasionally even in the tracheae.

P. tardicrescens has many morphological and cultural characteristics in common with *P. scleroteichum* (Drechsler, 1934), but is distinctly different from this form in its much slower growth rate and in the production of lobulate diverticula.

The English strain was isolated in November 1935 from Slough soil, *p*H 6·8, by means of wheat roots. The two strains approximate each other closely.

Description

Mycelium with a flat, radiate, non-lustrous growth on agar; hyphae mostly 2·5–5 μ. in diameter, and irregularly branched with fine laterals, knob-like appressoria being often present; radial growth on carrot maize-meal agar about 11 mm. in 24 hr. at 22° C.

Toruloid buds or a moderate development of lobulations infrequently produced in old cultures on the surface of plain agar containing wheat root tips, or in sterile water root-tip cultures, and observed intracellularly in living tissues; buds never complex, rarely exceeding 12 μ. in diameter; zoospore discharge not observed, instead, germ

Text-fig. 1. *Pythium tardicrescens.* Showing lobulate elements and sexual apparatus; drawn with the aid of a camera lucida. ×700. A. More or less toruloid lobulations within a root hair, with an external portion showing slightly more extensive development. B, C, E and F. Lobulate diverticula developed on old water-agar plates containing wheat root tips. D. The same as seen in the cortical cells of a wheat root in water culture. G, H, K. Oogonia with monoclinous antheridia. Note the characteristic lumpy nature of the oogonial contents. H, an intercalary oogonium. I. Diclinous apparatus. J, L, M. Oogonia with both monoclinous and diclinous antheridia. N. A mature oospore.

tubes are produced which may terminate in a dark conidium; spherical walls of germinated conidia frequently conspicuous in culture.

Oogonia smooth, terminal on short branches or rarely intercalary, commonly with large, lustrous oil globules, 17–30 μ. (average 24·1 μ.) in diameter, forming readily in plain agar containing wheat root tips or in water root-tip cultures, but more sparsely on the agar medium alone; empty oogonial "shells" common following failure to mature though occasionally oogonia become filled with tangled hyphae; delimiting septum frequently visible outside the edge of the oogonium. Antheridia up to six, but usually two to three; club-shaped or crook-necked, averaging 6–8·5 μ. in width, 10·5 μ. from apex to curve and 5·5 μ. from curve to septum, with a fertilization tube of 2 μ. and making fairly narrow to medium contact with the oogonium; they commonly arise from the oogonial stalk or a branch, but all may come from neighbouring branches, each of which may supply two or, less often, three.

Oospores, smooth, spherical or subspherical, usually free within the oogonium, 16–26 μ. (average 20·3 μ.) in diameter, with a central globule averaging 11·1 μ. usually clear and embedded in a finely granular matrix and a wall 1·25–2 μ.; they form best in solid or liquid cultures containing wheat root tips.

Causes a root rot of wheat in Saskatchewan, Canada, and in England; also pathogenic to other cereals and certain grasses when artificially inoculated; original isolation and type culture from diseased roots of *Triticum aestivum* L., Saskatchewan, 1929.

Pythium tardicrescens n.sp.

Mycelium, in agaro cultum, prostrate, sine fulgore et tarde crescens. Hyphae praecipuae plerumque 2·5–5 μ. latae, ramos angustiores et frequenter appressoria bullata gerentes; in culturis vetustis, etiam in radicibus Tritici vivis, toruloide ampliores, vel in diverticula subsimpliciter lobulata, ad 12 μ. lata, productae. Conidia globosa, opaca, in apice tubi singulatim orientia. Oogonia globosa, levia, in ramis brevibus acrogena vel rare intercalaria, guttulis oleosis magnis nitentibus repleta (nisi vero propter frustrationem vacua), 17–30 μ. in medio 24·1 μ. diam. Antheridia saepius 2–3, etiam 6, frequentius e stipite vel ramo oogoniali, rarius ex hyphis alienis, orientia, clavata vel cervicem curvata, prope 6–8·5 μ. lata, 16 μ. longa i.e. 10·5 μ. ex apice ad partem curvatam et 5·5 μ. ab hac ad septum; graciliter vel modeste cum oogonio conjuncta; tubo ubertatis 2 μ. lato praedita. Oosporae leves, globosae vel subglobosae, plerumque singulae intra oogonium ejus liberae, 16–26 μ., in medio 20·3 μ. diam., globulo centrali, saepius pellucido, in matrice granulari incluso, et exospora 1·25–2 μ. crassa praeditae.

Hab. Parasitica in radicibus Tritici aestivi L., prope Saskatchewan, Canada. 1929 (Typus); postea frequenter in Canada et in Anglia inventa.

Obs. A speciebus affinibus vel commixtis differt mycelio tardicrescenti diverticulis lobulatis praedito, et guttulis oogoniorum oleosis.

(5) *Pythium aristosporum* n.sp. (Pl. XXII, figs. 3, 5–7; Text-fig. 2)

This plant has been found in Canada only, being first isolated from diseased wheat roots in 1930, in Saskatchewan, and rarely encountered since. It is characterized by the ready production of abundant mature oospores in culture, the percentage of aborted oogonial remains being

small when compared with other congeners. Occasional peculiarities are the presence of a large bulge in the oospore wall (Pl. XXII, fig. 6, and Text-fig. 2 L), and two malformed oospores within one oogonial case, one spore being usually quite small and sometimes appearing as a disorganized lump of crushed protoplasm (Pl. XXII, fig. 7 and Text-fig. 2 K). It is closely allied to *P. graminicolum* and *P. myriotylum*, but in parallel cultures can readily be distinguished by the characteristics mentioned. Like *P. graminicolum* the collapsed oogonial wall with adhering antheridia persist for a considerable time after the oospores have matured (Text-fig. 2 H). Lobulate elements develop later in culture and are not as extensive as in those two species; germination by zoospores has not been induced. The antheridial elements more often arise in closer proximity to the oogonium than is the case in *P. myriotylum*, in comparison with which it also has larger oogonia and oospores. It is aggressively parasitic on the roots of wheat, and moderately so on oats, barley and rye.

Description

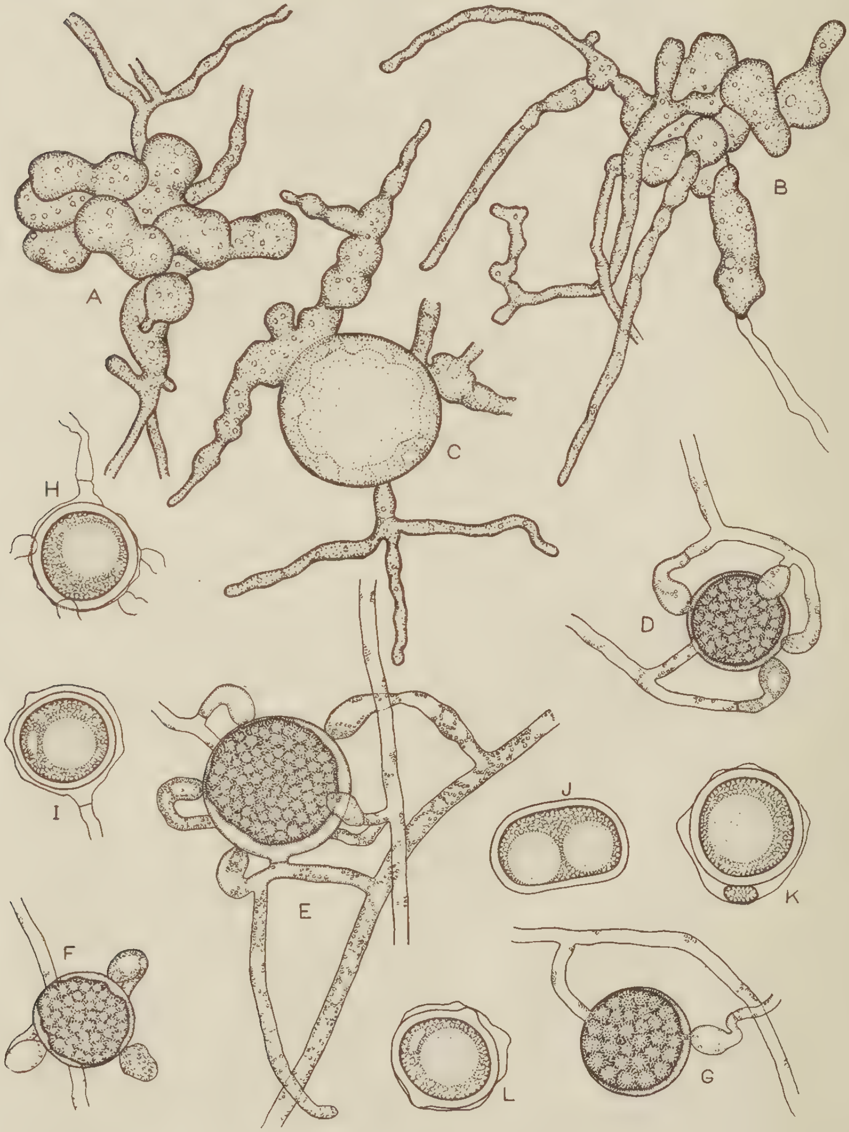
Mycelium somewhat lustrous with slight aerial development, soon becoming granular in appearance and creamy in colour, with hyphae 2·5–6·5 μ. in diameter and numerous appressoria; radial growth on carrot maize-meal agar 22–25 mm. in 24 hr. at 22° C.; conidia up to 40 μ. in diameter sometimes present, germinating by one or more germ tubes, irregular in course and soon branching.

Lobulations develop in due course in old water cultures; at first the individual elements are digitate, but in older complexes they are more often swollen lumps; germination is by numerous tubes, zoospore production not being obtained.

Oogonia smooth, subspherical, terminal, or intercalary, most often on short side branches, abundant, 21–36 μ. (average 28·8 μ.) in diameter; they form in 2–3 days on almost any media, both extra and intramatrically; septum usually some distance away from the oogonial wall, which persists long after maturation of the oospore. Antheridia usually three to six but as many as eight or more, club-shaped or crook-necked, moderately narrow, with narrow to medium contact, 6·8 μ. wide by 10·4 μ. from point of contact to curve and 6–7 μ. from curve to septum, frequently forming entanglements about the oogonium; a single branch may supply as many as four antheridia; vegetative prolongations from antheridia rarely observed; androgynous and diclinous.

Oospores smooth, subspherical, deep brown, usually free within the oogonium, abundant on most substrata and 13–30 μ. (average 24·2 μ.) in diameter, but with considerable range in size, with a central globule mostly 12–14 μ., a refringent spot 6 by 2·3 μ. and a wall about 1·5–2 μ.; germination by one or more germ tubes. Oblong oospores with as many as four reserve globules are occasionally observed in wheat roots, but more often oospores with one or two rounded lumps on their walls are present. Two oospores, either or both of which may be malformed, are sometimes encountered within a single oogonial case.

Isolated from diseased roots of wheat in Saskatchewan, Canada, 1930; also parasitizes other cereals and various grasses.



Text-fig. 2.

Pythium aristosporum n.sp.

Mycelium nonnihil nitens, minime aereum, mox granulare et sufflavum. Hyphae 2·5–6·5 μ. latae, appressoria numerosa gerentes; tempestive, in culturis aqueis, primo digitate- dein amorphe-lobulatae, zoosporis carentes, et tubis pluribus praeditae. Conidia 40 μ. diam., tubis germinalibus, singulis vel pluribus, vagis, mox ramosis instructa. Oogonia levia, subglobosa, plerumque a septo satis distanti limitata, acrogena vel intercalaria, frequentius in ramo brevi orientia; in omnibus mediis culturalibus per diem secundam vel tertiam numerosissime producta; membrana exteriori, longe post oosporae maturitatem manifesta, instructa; 21–36 μ., in medio 28·8 μ., diam. Antheridia 3–6, etiam 8, (ad 4 in ramo singulo orientia) androgyna et diclina, clavata vel cervicem curvata, modice tenuia, cum oogonio graciliter vel modeste conjuncta, 6·8 μ. lata, 17 μ. longa, i.e. 10·4 μ. ex apice ad partem curvatam, et 6–7 μ. ab hac ad septum; circum oogonium frequenter implicata. Oosporae leves, subglobosae, brunneae, singulae saepius intra oogonium ejus liberae, in substratis pluribus numerosae, plerumque 13–30 μ. in medio 24·2 μ., diam.; globulo centrali saepius 12–14 μ. diam., loculo refringenti 6 × 2·3 μ., et exospora 1·5–2 μ. crassa typice instructae. Oosporae in statibus abnormalibus interdum visae, (i) oblongae et 1–4 guttulatae (in radicibus Tritici vivis observatae); (ii) geminae, frequenter deformatae, in oogonio singulo locatae; (iii) tumoribus exosporae singulis vel duobus ornatae.

Hab. Parasitica in radicibus Tritici aestivi, prope Saskatchewan, Canada.

Obs. A speciebus affinibus P. graminicolo et P. myriotylo differt praecipue oogoniis maturis, in omnibus mediis culturalibus facile et numerose productis; minus et infrequentius, tumoribus exosporae et oosporis geminis.

(6) *Pythium torulosum* Coker & Patterson [?], (Coker & Patterson, 1927; Matthews, 1931; Van Luijk, 1934)

A species of *Pythium* in general agreement with the description of *P. torulosum* Coker & Patterson was isolated at least twice by Miss Mary D. Glynne, Rothamsted Experimental Station, from lesions on the stem bases of wheat plants in March 1936. The writer, about the same time, obtained it in pure culture from the roots of wheat seedlings from the same source, and somewhat earlier from the roots of seedlings from Reading. The English form is, also, probably in closer agreement with a fungus of the "*gracile*" group described by Petri (1930) as attacking mainly the basal portions of the stems of wheat plants in the province

Text-fig. 2. *Pythium aristosporum* developed in water culture containing wheat root tips; drawn with the aid of a camera lucida. × 700. A and B. Lobulate structures developed infrequently in old cultures germinating by germ tubes. C. A conidium germinating and producing ordinary germ tubes and swollen diverticula. D, E, F and G. Monoclinous and diclinous sexual apparatus. H. A mature oospore with persistent oogonial and antheridial remains. I. The same without antheridial remains. J. An oblong oospore with two central globules, developed in a root cell. K. A mature oospore with a flattened lump of protoplasm between it and the old oogonial wall. L. A mature oospore with two protuberances on the oospore wall; oogonial remains are still present.

of Padua, Italy, in 1930. In 1934, in the Netherlands, van Luijk (1934) obtained from grass roots a fungus which he regarded as *P. torulosum*.

English strain. Mycelial growth vigorous with tendency to develop aerially. Approximate radial growth rate on carrot-maize meal agar in 24 hr.: 8 mm. at 15° C., 13 at 20°, 15·5 at 25°, 4 at 30°, 0 at 35°. Sporangia made up of swollen or bulbous portions of the mycelium in a catenulate manner, seldom extensively digitate or lobulate. Zoospore discharge readily obtained. Zoospores 7·5 μ. average, when rounded. Oogonia (average 18·1 μ.), smooth, spherical, terminal or intercalary, form quickly in culture (second day). Antheridia one to two, more often only one, club-shaped, androgynous, arising usually from the oogonal stalk and disappearing after fertilization has been effected. Oospores abundant on most media, spherical, 16·8 μ. average, central globule 6·5–7·5 μ. average, with thick oospore wall, 2 μ. or more, completely filling the oogonium.

Van Luijk's culture from the Centraalbureau was studied comparatively with the English strain. Van Luijk's form has the tendency to produce a flat, rosette type of growth on rich media on agar plates, whereas the English form has a strictly radiate habit with a tendency to aerial development. Practically all types of relationships of antheridia to oogonia which have been found in one have also been observed in the other, though the types of relationships found most commonly in the respective forms differ markedly. It is characteristic of the English form for a single club-shaped antheridium to arise approximately 25 μ. down the oogonial stalk while, in van Luijk's form, it is usual for the antheridium to arise in closer proximity to the oogonium. The oospore in the English form averages 2·5 μ. larger, while the hyaline wall is twice (2 μ.) as thick as in van Luijk's form (1 μ.). Sporangia and zoospores are similar. The English form is slightly pathogenic to wheat, but van Luijk's form gave no evidence of parasitism. Van Luijk obtained a stimulation in the growth of grasses inoculated with his form (1934).

In comparison with the description and illustrations of *P. torulosum*, the English form rarely produces as complex lobulations and its oospore wall is much thicker (2 μ.) than that figured by Coker & Patterson. These authors remark on the thick nature of the oospore wall, but give only 0·8 μ. as the measurement. The oospores of the English strain show a closer resemblance to those figured by Matthews (1931, Pl. 6, fig. 5). In thickness of oospore wall van Luijk's form agrees well with Coker & Patterson's plant; the same is true for the oospore size. The oospores of Petri's form are somewhat larger, 16–24 μ., than any of the others. Petri did not obtain zoospore formation from the toruloid elements,

though this phenomenon is readily procured in both van Luijk's and our form.

The facts available seem to suggest that the English form is probably the same as the Italian. Both showed a tendency to attack the basal portion of the stems of wheat seedlings; van Luijk's form on the contrary was not pathogenic. The rosette habit of growth of van Luijk's form, together with the differences enumerated above, separate this form from the English one, to the extent of their being different varieties at least. It should be noted that neither Coker & Patterson (1927) nor Miss Matthews (1931) referred to a rosette type of growth in their plant. It is not an easy matter to settle the identity of our form under the circumstances, as neither the original type culture of *P. torulosum* nor that of Petri's fungus is available now. For the present it seems best to consider the English and the Italian forms as geographic strains of *P. torulosum*, even though it may broaden the concept of this species.

P. torulosum has not been recorded from Canada. When compared with the other species treated in this paper, the English strain is only slightly to moderately pathogenic to wheat seedlings.

Growth rates

Table I shows the comparative growth rates, in radial increase in millimetres during the second 24 hr. on carrot maize-meal agar plates, of the species of *Pythium* isolated from wheat.

Table I

Radial increment in millimetres in 24 hr. on carrot maize-meal agar plates

	Canadian strain					London, May, 1936 English strain				
Organism	15° C.	20° C.	25° C.	30° C.	35° C.	15° C.	20° C.	25° C.	30° C.	35° C.
Pythium arrhenomanes	9·5	18	22	24 +	Trace	10	17·5	22	25 +	Trace
P. graminicolum	—	—	—	—	—	6·5	10	11	0	0
P. volutum	9	12	13	Trace	0	9	12	13·5	Trace	0
P. tardicrescens	3·3	8	10	Trace	0	2	6·5	7·5	0	0
P. aristosporum	10	18	23	24 +	Trace	—	—	—	—	—
P. torulosum [?] (English strain)	—	—	—	—	—	9	13	15	3	0

Discussion

A striking fact in this investigation is the relative ease with which the better known *Pythium* species pathogenic to wheat could be obtained either from wheat seedlings in pots of soil collected at random from virtually any field with cereals in the rotation, or from seedlings collected

from the poorer looking parts of fields in March. It gives a probable indication of the wide distribution of these forms in the wheat fields of England. They are doubtless indigenous on native grasses and capable of attacking introduced varieties. Experiments conducted at the University of Saskatchewan have shown that species of fifteen genera of grasses are attacked when grown in naturally infested soil. The damage they cause in England is not known, but as the species of *Pythium* most pathogenic to wheat in other parts of the world have been shown to be present in five counties (Bucks, Cambs, Kent, Herts, and Lincs),[1] it is reasonable to infer that they account for a reduction in yields of wheat or other cereal which has, hitherto, been attributed to other causes. Of the six species, three are common to both Canada and England, namely, *P. arrhenomanes*, *P. volutum*, and *P. tardicrescens*; two were found in England and not in Canada, namely, *P. graminicolum* and *P. torulosum* [?], and one in Canada only, *P. aristosporum*.

Another noteworthy fact is that the six species under discussion belong to the group with lobulate sporangia. Numerous sphaerosporangial forms were isolated, but these invariably proved non-pathogenic or weakly pathogenic. These latter fail to produce brown discoloration and necrosis of the root tissues; they do, however, cause a retardation of growth in the length of the main seminal roots, with the subsequent development of an excessive number of fine laterals. It is possible that this is due to some toxic product excreted by the fungus (cf. Vanterpool, 1933). If this should be so, their presence in the soil may increase the liability of the roots to attack by the parasitic species themselves.

By the method of isolation used in these investigation in England and noted earlier in this paper, 65–90% of the isolates from discoloured root lesions are species of *Pythium* and the remainder mainly *Fusarium* spp. Of the *Pythium* isolates 30–50% are parasitic and may belong entirely to one species or to as many as three, depending on the source of the wheat seedlings. Usually, a given sample of seedlings from the field yielded two or three parasitic species, while the sample from pots gave one or two parasitic species. This demonstrates that in a comprehensive study of the fungal flora of the roots of any crop plant, use should be made of a method, such as the one employed in this investigation, of obtaining in culture the phycomycetous fungi present; otherwise, the forms obtained in culture are not representative of those occurring in the roots.

[1] More recently, May 1937, Mr S. D. Garrett, Rothamsted Experimental Station, in a written communication informed me of *Pythium* damage to wheat seedlings in Yorkshire during March, following a wet period.

It is not an easy matter to assess the parasitic vigour of the various species because of the difficulty of obtaining uniform distribution of the active inoculum throughout the soil. This is particularly true of forms such as *P. tardicrescens*, which are more limited in their cultural requirements but which, under their optimum conditions, may produce symptoms strikingly like those produced under field conditions (Pl. XXII, fig. 2). Experience has shown that the preliminary laboratory tests on parasitism conducted in small flasks, as described previously, give as trustworthy an indication of relative parasitic ability as do more comprehensive experiments in artificially inoculated soil.

It is clear from a review of the literature that *P. arrhenomanes* and *P. graminicolum* attack many graminaceous hosts in both tropical and temperature climates. Doubtless, the finding of one or both of these species on wheat seedlings in the field in large wheat-growing countries, such as Australia and the U.S.A., where they have not hitherto been reported on wheat, merely awaits the search.

In England, by far the greater part of the wheat crop is winter wheat while, in those sections of the Canadian prairie provinces where the disease is common, only spring wheat is grown. This, together with the extreme differences in climate, makes it probable that the factors predisposing the plants to attack by the root parasites in the two countries differ not only in degree but also in kind. If, as has been contended (Carpenter, 1928, 1934; Cooke, 1934; Vanterpool, 1935), deficient or unbalanced nutrients may predispose plants to attack by *Pythium*, the use of fertilizers for a given soil type may alleviate the damage.

SUMMARY

The results of a comparative study of six species of *Pythium* pathogenic to wheat seedlings are presented. *P. arrhenomanes*, *P. volutum*, and *P. tardicrescens* n.sp., were found in both Canada and England; or, for *P. graminicolum* and *P. torulosum* [?] in England and not in Canada; and *P. aristosporum* n.sp., only in Canada. The English forms were obtained from Bucks, Cambs, Kent, Herts, Berks, and Lincs, and the Canadian forms from Saskatchewan. The similarity of the English form considered as a geographic strain of *P. torulosum* with the form reported on wheat in Italy by Petri is pointed out. Attention is drawn to the wide geographic distribution of species of *Pythium* on graminaceous hosts. By the method of isolation used, it was relatively easy to obtain one or more of these fungi either from wheat seedlings grown in potted soil collected at random from virtually any field with cereals in the rotation, or from

seedlings collected from the poorer looking parts of such fields. All of the parasitic species isolated belong to the group of the genus with lobulate sporangia. It is suggested that the many sphaerosporangial forms encountered may render the wheat seedlings more liable to attack by the pathogenic forms themselves. The use of fertilizers may prove beneficial.

Acknowledgements

Appreciation is expressed to Prof. W. Brown for his interest in the work, for his many courtesies and for the use of the facilities of his laboratory at the Imperial College of Science and Technology; to Mr S. F. Ashby, Director of the Imperial Mycological Institute, for his invaluable help, for kind advice, for facilities granted, and for a critical reading of the manuscript. I am much indebted to Mr E. W. Mason for writing the descriptions in Latin of the two new species, to Mr W. Buddin, Dr G. W. Padwick and Mr R. V. Tipler for supplying plant material, to Miss Mary D. Glynne for cultures, and to Mr S. D. Garrett and Dr J. B. Marshall for soil samples.

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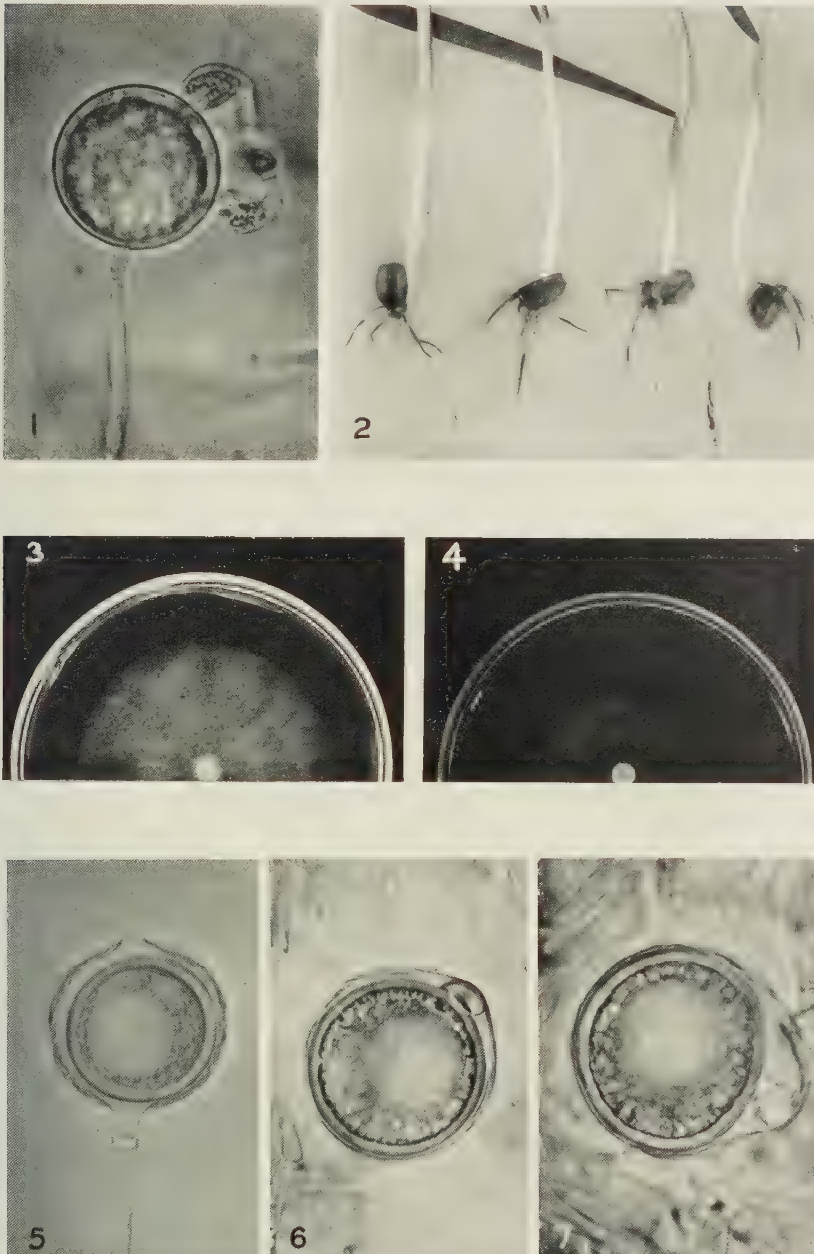

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EXPLANATION OF PLATE XXII

Fig. 1. *Pythium tardicrescens.* Oogonium with two diclinous antheridia arising from a single stalk, in a water culture containing sterile wheat root tips. ×900.

Fig. 2. Wheat seedlings, 4 weeks old, showing the characteristic root-tip lesioning following inoculation of sterilized soil with *P. tardicrescens.*

Fig. 3. A 2-day culture of *P. aristosporum* on carrot maize-meal agar, showing tendency for slight aerial development.

Fig. 4. A 2-day culture of *P. tardicrescens* on carrot maize-meal agar, showing the flat, radiate, non-lustrous growth.

Figs. 5, 6 and 7. *P. aristosporum* on water-agar containing wheat root tips. ×900. Fig. 5. A typical, mature, intercalary oospore, not filling the oogonial cavity. Fig. 6. An oospore with a protuberance on its wall. Fig. 7. A disorganized mass of protoplasm is to be seen between the oospore and the remains of the oogonial wall.

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FURTHER OBSERVATIONS OF THE INCIDENCE OF BLOTCHY RIPENING OF THE TOMATO

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(With 7 Text-figures)

CONTENTS

I. Introduction

Blotchy ripening of tomato fruits has been described and illustrated by Bewley & White (1926), who showed that this disorder, prevalent on plants grown in unmanured soil, is associated with a deficiency of nitrogen and potassium, especially the latter. The inverse relationship demonstrated between the percentage of blotchy fruit and the increments of potash and nitrogen added to the soil of plots deficient in these nutrients, provides strong evidence that the factors controlling the incidence of blotchy ripening are mainly nutritional.

Seaton & Gray (1936) consider that nutrient deficiency in relation to this disorder is "secondary and occasional" and advance the hypothesis that the primary cause of blotchy ripening is a "water-deficit" leading to sudden withdrawal of water from the fruit and mechanical disruption. They base this supposition on their interpretation of the morphological appearance in section of affected fruits, and do not present any experimental evidence to support their views. Robbins (1937) has recently

shown that a "water-deficit" leads not to blotchy ripening but to a separate physiological disorder—blossom end-rot.

II. THE EFFECT OF LIGHT ON THE INCIDENCE OF BLOTCHY RIPENING[1]

Table I (compiled from the Cheshunt Experimental Station *Annual Reports*) and Fig. 1, give the annual variations in proportion of blotchy fruit from plants grown during the period 1921–9 on plots from which nitrogen and potassium, respectively, were omitted continuously from the scheme of manuring. Fig. 1 shows that the annual fluctuations of one plot correspond with those of the other. It follows that there must be a third factor, affecting both plots, involved in the production of

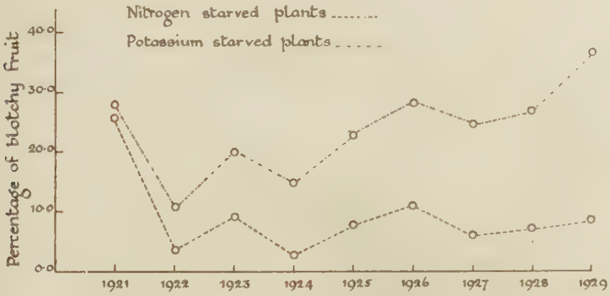

Fig. 1. Annual fluctuation in percentage of "blotchy" fruits of (*a*) potassium-starved plants, and (*b*) nitrogen-starved plants.

blotchy fruit. This factor is probably light, since the value of a correlation coefficient between the percentage of blotchy fruit on the nitrogen-starved plot and the mean daily number of hours of sunlight between 1 April and 31 August for the years 1922–7 inclusive is $-0{\cdot}803$ ($P=0{\cdot}03$). This negative association seems definite, and contrasts with the aberrant year 1921 when blotchy fruits were exceptionally prevalent in spite of an abnormally high number of hours of sunshine. Similar relationships, complicated by a rising trend in the proportion of blotchy fruit with time, are apparent in Fig. 1 for the fruit of the potassium-starved plants.

It is of interest to note that a definite positive correlation between blotchy fruit and high temperature cannot be traced. The correlation coefficient between daily maximal temperature and the values for blotchy fruit that are shown above to be correlated with light is $+0{\cdot}346$, a

[1] This and the following section are revised versions of parts of a thesis (unpublished) presented to the University of Cambridge in partial satisfaction of requirements for a Diploma in Horticultural Science.

statistically insignificant value. If the period between 1 June and 31 July when the influence of temperature on the water-relations of the fruit should be most marked is considered, the correlation coefficient is only +0·206. The correlation that may be traced between blotchy ripening and light confirms the view that this disorder is nutritional, whereas the low correlation coefficients between blotchy ripening and high temperature militate against the hypothesis of Seaton & Gray that blotchy ripening is primarily due to a water-deficit.

Table I

Annual variation in percentage of "blotchy" fruits

	Mean daily no. of hours of sunshine 1 April to 31 Aug.	Potassium-starved plants	Nitrogen-starved plants
1921	7·02	28·0	25·8
1922	6·25	10·8	3·7
1923	5·93	20·3	9·5
1924	6·22	15·1	2·9
1925	5·92	24·0	7·8
1926	5·47	28·5	11·3
1927	5·67	24·9	6·2
1928	5·95	26·8	7·3
1929	6·39	36·8	8·7

III. The differential effect of light on weight of fruit of potassium-starved plants

The weights of fruit from plants grown on plots, on which nitrogen and potassium, respectively, were omitted from the scheme of manuring over a period of 14 years, have been recorded in the Cheshunt Experimental Station *Annual Reports* from 1916 to 1929. These values have been discussed by Bewley (1929) who points out that the crop of the potassium-starved plants is relatively high in 1921, and suggests that the effect of light is equivalent to that of potassium in relation to nitrogen supply, so that in a sunny season nurserymen should manure with less potassium and more nitrogen. Bewley, referring to crop records from blocks with comparable manurial treatment over a period of many years, concludes "the yield per acre varies directly in relation to the total hours of bright sunshine 1 April to 30 September".

The nutrient deficiency experiments originally consisted of plots with duplicate treatments in adjacent houses. The treatments remained comparable until 1924, when the soil of one house was sterilized by steam, while the crop of the completely manured plants in the other shows, subsequently, the ageing effect usual in the continual cropping of glass-

house soils. Available data for 1916 are incomplete. Estimation of the error of the treatments is, therefore, facilitated by confining the discussion to the years 1917–23 inclusive.

Table II gives the crop in lb. per plant for the nitrogen-starved, potassium-starved, and completely manured plants. Since different varieties were used in the two houses the total weights of crop are not directly comparable. This difficulty may be overcome by calculating for each season the weight of crop of the nitrogen-starved and potassium-starved plants as a percentage of that of the completely manured plants. Fisher's analysis of variance has been performed on these relative values (columns 5, 7, 10 and 12 of Table II) and the results are given in Table III. Table III shows that the effects of season, treatment, and their interaction are significantly greater than the experimental error as estimated from duplication of the treatments. The outstanding feature of Table III is the high degree of significance of the interaction between treatment and season, and this effect outweighs that of treatment. This interaction must be due to a differential effect on potassium-starved and nitrogen-starved plants of some factor which is highly characteristic of seasonal

Table II

Weight of fruit in lb. per plant of completely manured plants (C.A.), *nitrogen-starved plants* (−N), *potassium-starved plants* (−K)

	Mean daily no. of hours of sunshine	House 1					House 2				
	1 April to 31 Aug.	C.A.	−N	Relative	−K	Relative	C.A.	−N	Relative	−K	Relative
1917	6·27	5·11	5·60	109·6	4·65	91·0	5·64	6·08	107·8	5·18	91·8
1918	5·92	3·32	3·62	109·0	2·76	83·1	4·12	4·22	102·6	3·39	82·4
1919	6·28	5·57	5·98	107·4	4·95	88·9	5·86	6·34	108·3	5·46	93·3
1920	5·50	3·45	3·57	103·4	3·11	90·1	4·94	4·45	90·1	4·29	86·9
1921	7·02	4·08	4·27	104·7	4·65	114·0	5·84	5·54	94·8	5·74	98·2
1922	6·25	4·13	3·47	85·2	3·96	95·9	5·43	5·04	92·7	5·31	97·8
1923	5·93	5·09	4·69	92·1	4·51	88·6	5·40	4·70	87·1	4·69	86·8

Table III

Analysis of variance of annual fluctuation in crop of nitrogen-starved and potassium-starved plants

	Degrees of freedom	Variance	"Z"	1% point	5% point
Treatment	1	393·82	**0·709**	—	0·523
Season	6	102·59	**1·382**	1·091	—
Treatment × season	6	149·81	**0·890**	0·747	—
Remainder (error of duplication)	14	24·83	—	—	—

changes. Such factors are sunlight, temperature and rainfall. The effect of rainfall is discounted under glass, while the relative magnitude of the correlations with quality of fruit (p. 546) suggests that temperature does not have such a marked influence on fruiting as light.

Fig. 2 shows the annual variation in crop of the completely manured plants, together with the mean daily number of hours of sunlight from 1 April to 31 August, a period selected (White, 1925) as roughly corresponding with the period of fruiting of the tomato. The correlation coefficient between hours of sunlight and weight of crop is +0·438, a value which does not reach the conventional level of significance.

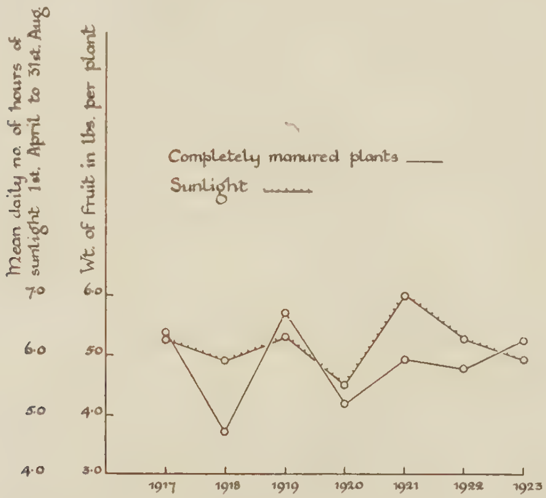

Fig. 2. Annual fluctuation in weight of fruit of completely manured plants together with the mean daily number of hours of sunlight from 1 April to 31 August.

Relative values of the crop of the potassium-starved and nitrogen-starved plants (Table II) are plotted in Figs. 3 and 4, in comparison with the daily number of hours of sunlight. The correlation coefficient between the relative values for the crop of the potassium-starved plants and light is +0·846, and this association must be considered definite since it would have occurred by chance in less than 2% of random cases. The crop of the nitrogen-starved plants falls throughout the period and it is necessary, therefore, to calculate correlation coefficients with time:

Nitrogen and light	...	$r_{12} = +0{\cdot}172$
Nitrogen and time	...	$r_{13} = -0{\cdot}922$
Light and time	...	$r_{23} = +0{\cdot}062$

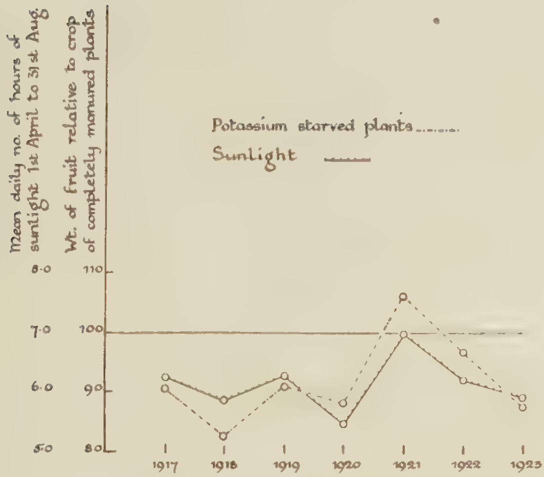

Fig. 3. Annual fluctuation in weight of fruit of potassium-starved plants relative to the crop of completely manured plants, together with the mean daily number of hours of sunlight from 1 April to 31 August.

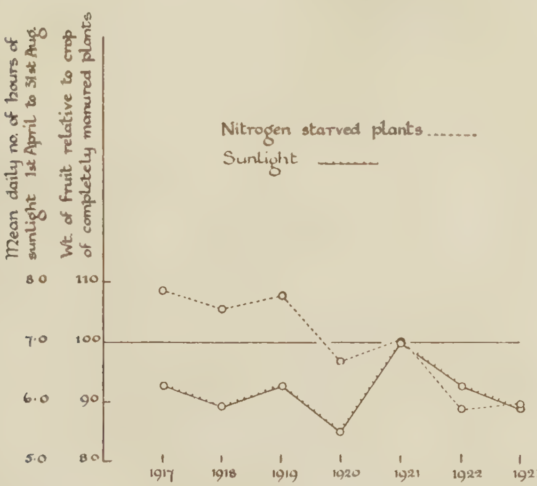

Fig. 4. Annual fluctuation in weight of fruit of nitrogen-starved plants relative to the crop of completely manured plants, together with the mean daily number of hours of sunlight from 1 April to 31 August.

The partial correlation coefficient between nitrogen and light, eliminating the effect of time, is $+0{\cdot}587$. Although this correlation is higher than that characterizing the completely manured plants, it falls short of the level necessary to establish a definite association. It is concluded that the relationship between weight of crop of completely manured or nitrogen-starved plants and light is not of sufficient magnitude to be established by the data considered. In the potassium-starved plants, on the other hand, there is a close relationship between weight of crop and the level of the light factor.

Table IV

Effect, relative to potassium-starved plants, of increasing potassium supply

% of K_2O in fertilizer	Weight of fruit	"Blotchy" fruit
0	100·0	100·0
2½	102·5	45·1
5	118·3	31·6
7½	119·9	42·4
10	121·2	27·1
12½	124·3	24·8

Table IV summarizes the effect of increasing potassium supply on weight of fruit and on the proportion of blotchy fruit of potassium-starved plants. It is clear that a significant increase in crop and decrease in the proportion of blotchy fruit can be obtained *either by increase of potassium supply or by increase of light.*

IV. The effect of potassium on ripening

To elucidate the mode of effect of potassium in the production of blotchy fruit, reference may be made to the chemical changes taking place during ripening. These have been investigated by Sando (1920) who reaches the following conclusions: "The most striking change during ripening is that undergone by carbohydrates. In the first stage analysed it was noticed particularly that insoluble carbohydrates composed 52·1 % of the total carbohydrates present, while in the last stage, that of ripe fruit, soluble carbohydrates were in excess, amounting to 77·3 % of the total. Nearly all of the total sugar in the tomato fruit is apparently invert sugar and this increases from 25·56 % in the case of 14-day-old fruit to 48·32 % in ripe fruit, an increase of 89 %. Starch decreases during maturation from 15·84 to 2·65 %. The most marked decrease, as would be expected, is noticed during the period of transition from green to red." These observations suggest that failure of the blotchy areas of

fruit to ripen is associated with failure to convert starch into sugar. In order to test this hypothesis, portions of the carpel walls of normal fruits from the completely manured plants and blotchy fruits from the potassium-starved plants were cut out and grouped into samples of equal wet weight. Juice was extracted by pressure and equal volumes from both sets of samples were centrifuged. An ascending series of volumes of the juice obtained was mixed in a set of hard glass test-tubes with a descending series of volumes of a standard starch solution. Drops were extracted from time to time as indicators. After a few hours dilute iodine was added to each tube. *It at once became apparent that concentrations of starch that had been hydrolysed by the juice from the completely manured fruits were not being hydrolysed by the juice from the potassium-starved fruits.* Similar tests with "blotchy" and "normal" areas from the same fruits gave similar results. It is concluded that the unripened areas of the fruits of potassium-starved plants are characterized by low amylolytic activity. That blotchy ripening occurs in lesser degree in association with nitrogen starvation is of interest in view of the general belief that enzymes, including amylase, are of protein composition. Repetition of the experiment with substitution of "greenback" for "blotchy" fruits led to similar results.

V. CARBOHYDRATE ACCUMULATION IN LEAVES OF POTASSIUM-STARVED PLANTS

In order to obtain an indication of the effect of potassium on the translocation of carbohydrates from the leaves, changes in dry weight per unit area associated with potassium starvation were compared with corresponding changes with full nutrient supply and with nitrogen starvation. Since it was impracticable to use whole leaves the area of corresponding leaflets was estimated by enclosing the leaves between thin panes of glass, drawing an illuminated outline on tracing paper and tracing the areas with a planimeter. The leaves were subsequently dried at 100° C. and weighed. The work had to be limited to comparison of a single plant from each treatment. Care was taken to select a normal completely manured plant, and plants with characteristic though moderate symptoms of nitrogen and potassium starvation. The terminal pair of leaflets of every leaf on the plants was used. The results are shown in Table V. The dry weight per unit area of the nitrogen-starved plant is consistently high and the effect is visible even in the youngest leaf. The dry weight per unit area of the completely manured and potassium-starved plants are plotted in Figs. 5 and 6. The dry weight per unit area

of the completely manured plant increases from the youngest to the sixth leaf from the growing point, but remains relatively constant or

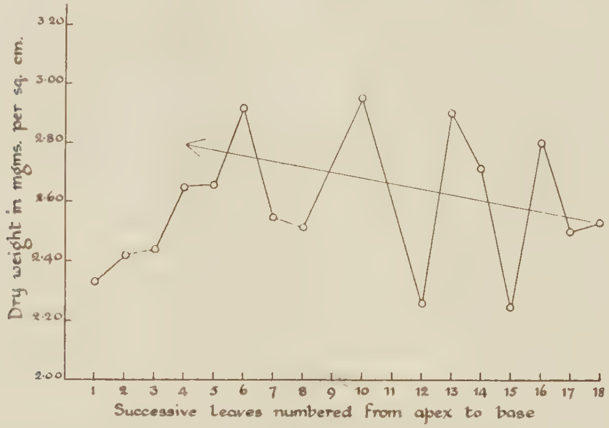

Fig. 5. Dry weight per unit area in mg. per sq. cm. of corresponding leaflets of successive leaves, numbered from apex to base, of a completely manured plant.

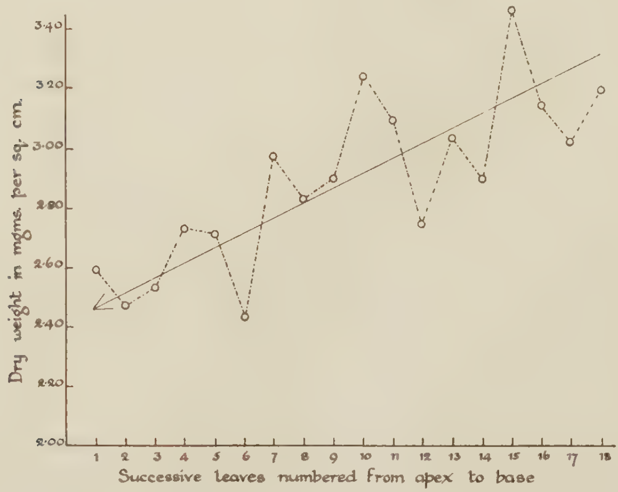

Fig. 6. Dry weight per unit area in mg. per sq. cm. of corresponding leaflets of successive leaves, numbered from apex to base of a potassium-starved plant.

falls slightly in older leaves. The dry weight per unit area of the leaflets of the potassium-starved plant *continues to increase from the youngest to the oldest on the plant.*

Table V

Dry weight (mg. per sq. cm.) of corresponding leaflets from every leaf (numbered from apex to base) of a typical plant from each treatment

Leaf no.	Completely manured			Nitrogen-starved			Potassium-starved		
1	2·29	2·33	2·37	2·72	2·74	2·77	2·61	2·59	2·57
2	2·33	2·42	2·51	2·91	2·94	2·97	2·57	2·47	2·37
3	2·48	2·44	2·39	2·99	3·01	3·04	2·53	2·53	2·54
4	2·67	2·65	2·62	2·97	2·99	3·02	2·86	2·73	2·60
5	2·70	2·66	2·62	3·28	2·27	3·25	2·75	2·71	2·66
6	3·00	2·92	2·84	3·14	3·18	3·22	2·34	2·43	2·52
7	2·65	2·55	2·44	2·98	2·98	2·97	3·01	2·97	2·93
8	2·44	2·51	2·58	3·12	3·01	2·91	2·87	2·83	2·79
9	2·95	2·95	2·96	2·41	2·49	2·58	2·88	2·90	2·92
10	2·30	2·26	2·22	2·61	2·58	2·54	3·22	3·24	3·26
11	2·86	2·90	2·94	2·67	2·67		3·12	3·09	3·05
12	2·75	2·71	2·66	2·67	2·66	2·65	2·75	2·74	2·73
13	2·22	2·24	2·26	2·58	2·65	2·72	2·89	3·03	3·18
14	2·81	2·80	2·78	—	—		2·89	2·89	2·90
15	2·32	2·50	2·69	—	—		3·80	3·46	3·11
16	2·71	2·53	2·35	—	—		3·10	3·14	3·18
17	—	—		—	—		3·15	3·02	2·88
18	—	—		—	—		3·31	3·19	3·07

To demonstrate further that severity of potassium starvation affects carbohydrate accumulation in the leaves, four plants were selected from the no-potassium plot. These plants were ranged in order according to severity of starvation symptoms. Differences between the two plants with most severe starvation symptoms were slight and have been averaged in plotting the results. These values, shown in Table VI and Fig. 7, confirm that increasing severity of potassium starvation is associated with progressive increase in level of dry weight per unit area.

Table VI

Dry weight (mg. per sq. cm.) of corresponding leaflets of leaves (numbered from apex to base) of plants showing (a) slight, (b) moderate, (c) and (d) severe symptoms of potassium starvation

	(a)		(b)		(c)		(d)	
1	3·57 3·67	3·62	4·06 3·68	3·87	4·33 4·69	4·50	4·12 4·12	4·12
2	3·62 3·46	3·54	4·27 4·40	4·34	4·66 5·38	5·02	4·88 4·22	4·55
3	3·13 3·25	3·19	4·19 4·12	4·16	4·86 5·23	5·05	5·11 5·09	5·10
4	3·06 3·19	3·13	3·90 3·92	3·91	4·36 4·31	4·34	5·00 4·89	4·95
5	2·81 —	2·81	3·61 4·02	3·82	3·35 3·64	3·50	4·23 4·06	4·15
6	3·30 3·35	3·33	4·09 —	4·09	4·26 3·58	3·92	5·17 5·04	5·11

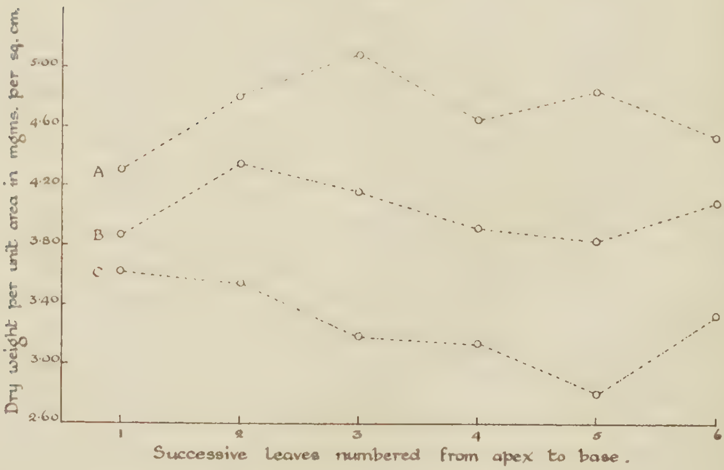

Fig. 7. Dry weight per unit area in mg. per sq. cm. of corresponding leaflets of successive leaves numbered from apex to base of plants showing (A) slight, (B) moderate, and (C) severe symptoms of potassium starvation.

VI. The histology of blotchy fruit

A histological examination of the affected tissues of blotchy fruit was carried out (White, 1925) and the following conclusions were reached:

(1) Blotchy ripening is not invariably associated with structural disorganization of the tissues, which appears to follow rather than precede failure to ripen. Extensive plugging may occur, especially in

the cells of the phloem, but also, occasionally, in the woody elements, without obvious structural damage to either of these tissues or adjacent parenchyma.

(2) The membranous lining of the cavities adjacent to necrotic bundles is derived in part from disorganized parenchyma and in part from the phloem, the cross-walls of these cells disintegrating and the longitudinal walls thickening extensively.

These observations are not in complete agreement with those of Seaton & Gray (1936) who claim that the vascular bundles of blotchy fruits are unaffected, but appear necrotic because of the draping around them of the broken-down cell debris of adjacent parenchymatous tissue.

VII. DISCUSSION

Increase in number of hours of sunlight is associated with decrease in percentage of blotchy fruit despite a tendency to increase in weight of crop, which should be associated with increased consumption of nutrients and, therefore, with greater intensity of potassium and nitrogen starvation. This suggests that blotchy ripening is not due directly to lack of potassium or nitrogen but to metabolic changes that are counteracted by increase of light.

The increase in weight of crop associated with increase of sunlight is statistically significant, under the experimental conditions, only in the case of the potassium-starved plants. Since previously observed effects of potassium deficiency on fruiting—notably acceleration of blossoming and prolongation of the ripening period (White, 1938)—are associated also with low carbohydrate level, independently of potassium supply, it is reasonable to attribute the beneficial effect of increase of light on weight of fruit and proportion of blotchy fruit of potassium-starved plants to increase in carbohydrate level.

Increasing severity of potassium starvation is association with progressive increase in level of dry weight per unit area of the leaves. This accumulation of surplus carbohydrate in the leaves of potassium-starved plants, in conjunction with the similarity between the effects of potassium deficiency on fruiting and those of plants with relatively low carbohydrate level, favours the view that translocation of carbohydrates is impaired in potassium-starved plants. In support of this view may be cited the experiments of Phillips *et al.* (1934) who show that the starch and dextrin content of potassium-deficient tomato plants is high in the leaves but low in the stems.

The juice of the affected areas of blotchy fruit is characterized by low amylolytic activity; moreover, the cell walls of the phloem of the vascular bundles of affected areas of blotchy fruits may be characterized by extensive thickening. It is now generally accepted that the phloem is the sugar-conducting tissue of plants, and it may well be that sugars are being condensed to cellulose in the vascular bundles of blotchy fruits of potassium-starved plants, instead of participating in the normal processes of ripening.

These observations are conformable with the view that blotchy ripening is symptomatic of deranged carbohydrate metabolism, the fruits of potassium-starved plants being characterized by abnormal carbohydrate changes, while translocation of carbohydrates from the leaves to the fruits is impaired, possibly owing to the occurrence of similar changes in the leaves. It may well be that these changes are accompanied by derangement of the water relations, leading to the association of blotchy ripening with disruption of fruit tissues, since the prevalence of Leaf Scorch on potassium-starved plants (Bewley & White, 1926) suggests lack of balance between water supply and water loss.

VIII. Summary

1. The annual fluctuation in percentage of fruit affected with "blotchy ripening" on potassium-deficient and nitrogen-deficient plots at the Cheshunt Experimental Station is significantly negatively correlated with the mean daily number of hours of bright sunshine between 1 April and 31 August.

2. The weight of fruit of potassium-deficient plants is raised and the percentage of blotchy fruit reduced, either by increase of potassium supply or by increase of light. This beneficial effect of increase of light on the crop of potassium-deficient plants is of much greater magnitude than any corresponding effect on the crop of nitrogen-deficient or completely manured plants.

3. The juice of the blotchy areas of potassium-starved fruits has diminished capacity for starch hydrolysis.

4. Increasing severity of potassium starvation is associated with progressive increase in level of dry weight per unit area of the leaves.

5. The influence of potassium on the production of blotchy fruit is briefly discussed.

The author is indebted to Dr W. F. Bewley for permission to publish this paper.

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PHYSIOLOGICAL RELATIONSHIPS BETWEEN INSECTS AND THEIR HOST PLANTS

I. THE EFFECT OF THE CHEMICAL COMPOSITION OF THE PLANT ON REPRODUCTION AND PRODUCTION OF WINGED FORMS IN *BREVICORYNE BRASSICAE* L. (APHIDIDAE)

By A. C. EVANS

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(With 4 Text-figures)

CONTENTS

I. Introduction

Much work has been carried out upon the biological relationships existing between insects and their host plants (Davidson, 1925; Painter, 1936; Trouvelot *et al.*, 1933). It has been established that different species and varieties of host plant, manurial conditions, etc., may have great effects upon the rate of reproduction, rate of growth and longevity of the insects feeding upon them, but little study has been made of the factors in the plant that bring about these effects, i.e. whether they are due to variations in the general nutritive level of the plant, presence or absence of specific substances giving a suitable or unsuitable taste, presence of poisons, lethal proteins, absence of vitamins or salts. Painter gives an interesting and extensive discussion of the types of relationship which are likely to exist between insects and plants, at the same time emphasizing the paucity of exact chemical data in existence.

The insect chiefly used in the present work is the cabbage aphis, *Brevicoryne brassicae* L. It has many advantages for this type of work; many generations can be bred in a short time and small space and it has a wide variety of host plants (cf. Petherbridge & Mellor, 1936). The effect of the host on the production of apterous, alate and sexual forms can be studied and its common food-plant, cabbage, can easily be grown. It has the marked disadvantage, however, that its principal food, the contents of the phloem tubes, cannot be extracted for analysis. Therefore, it has been necessary to analyse the leaf lamina and to use its chemical composition as the closest approximation obtainable at present. On account of the size of the aphis, it is very difficult to obtain adequate quantities of a uniform age for chemical analysis. Davidson (1925) suggested that the manurial treatment of the plant has a definite effect in increasing the percentage dry weight of *Aphis rumicis* L. and, therefore, the absolute nitrogen content. However, there is clearly something incorrect in his table since he records a dry weight of 0·215 g. per individual aphis, an improbable figure for this species.

II. Methods of analysis

The plant material was preserved for analysis in the following manner: the leaves were divided into two portions lengthwise, discarding the midrib, one half of the sample was put into boiling 95% alcohol, boiled for a few minutes and preserved in this; the other half was dried at 60° C. to constant weight. Analyses of soluble sugars and starch were carried out on the material preserved in alcohol, and the dried material was used for dry weight determinations and estimation of nitrogen.

Nitrogen. The unmodified Kjeldahl method was used. Non-protein nitrogen was extracted by grinding up the material in 2·5% trichloracetic acid in 0·02% phenol for 20 min. and filtering. The insoluble nitrogen of the residue is termed protein nitrogen (Richards & Templeman, 1936).

Soluble sugars. The soluble sugars were extracted by boiling the material in 80% alcohol for 8 hr., filtering, and evaporating off the alcohol under reduced pressure at 35° C., the residue being diluted with distilled water and cleared with a few drops of dibasic lead acetate as prepared by Van Plank (1936). Excess lead was removed with 3·1% disodium hydrogen phosphate. An aliquot of the cleared solution was acidified with hydrochloric acid to 0·4% and heated on a water-bath at 70° C. for 15 min. to hydrolyse any sucrose present. The solution was then

neutralized and the sugars estimated by the method of Shaffer & Somogyi (1933).

Starch. The starch was extracted from the sugar free residues by boiling them for 15 min. in 95% alcohol to which 1 ml. of concentrated hydrochloric acid per 100 ml. had been added (Hanes, 1936). The acid alcohol was filtered off and the starch extracted by boiling the treated material in 20 ml. of water for 20 min. Two extractions sufficed. The starch was hydrolysed in 2·5% hydrochloric acid for 2 hr. on a boiling water-bath. The amount of sugar present was estimated by the Shaffer-Somogyi method after neutralization.

III. The influence of the chemical composition of the host plant on reproduction

Two experiments were carried out which enable the effects of the nitrogen and carbohydrate contents of the host plant on reproduction to be studied. One experiment was expressly designed for this study while the other, designed for another purpose, gave results of interest on these points. A wide variation in the chemical composition of the host plant used (Sutton's cabbage, variety Tender and True) was obtained by growing one set of six plants under cellophane cages and another set of six plants under cellophane cages covered with two layers of Courtauld's black art silk voile which cut out 80% of the light. The voile covers were placed on the cages 3 days before the insects were placed on the plants. On 25 August 1936 each plant was infested with three young apterous aphides born of winged parents. Reproduction commenced on 27 August and the experiment was terminated on 5 September. Table I shows the number of aphides produced on each plant.

Table I

Number of young produced by three apterae in 10 days under light and dark conditions of illumination of the host plant

Cage ...	1	2	3	4	5	6	Mean
Light	58	51	77	77	59	84	68
Dark	4	1	15	26	2	23	12

The difference in the rate of reproduction of the aphides fed on plants grown under normal light conditions and those grown under subnormal conditions of light is clearly significant. This experiment is referred to below as Exp. 1. In view of the work of Shull (1930) on the influence of light on the production of winged forms, it was suspected that the direct influence of light upon the aphides themselves might

have affected the rate of reproduction. This however did not prove to be so, as aphides placed in smaller cages under the above conditions of light on the petioles of cabbage leaves, the laminae of which were exposed to full light, did not show any significant difference in their rate of reproduction. The mean number of young produced by six sets of three apterous aphides under normal light conditions was ten, while under subnormal light conditions it was fourteen.

The second experiment consisted of six plants under cellophane and six under perforated zinc covered with cellophane. The plants were divided into six blocks, each block consisting of one pair of dissimilar cages. The cellophane cages in each block were infested, at random, with a number of aphides varying from one to six and the corresponding zinc cage of each pair with double the number. This experiment was primarily designed to study the effect of the chemical composition of the host plant on the production of alatae, and Davidson (1925) has shown that the rate of reproduction of *Aphis rumicis* on beans was about halved by growing them under perforated zinc cages. In this experiment the plants growing under the zinc cages were therefore infested with twice as many individuals as those growing under cellophane cages in order to eliminate as far as possible any effects of overcrowding on the production of alatae. The plants were grown in a shady insectary and were infested on 21 July 1936 with nearly full grown apterae born of apterous parents. Reproduction was occurring on all plants on 24 July and the experiment was terminated on 14 August. All the parent aphides were alive on 27 July and each had produced about ten young; there was no difference between the rates of reproduction in the two sets of cages at this date but marked differences had appeared when the experiment was terminated. The mean number of young descended from one female under light conditions was 445 and under the darker conditions was 160. The variations about the mean were great in each case but the difference is significant when examined by the t test, $P=0{\cdot}01$. This experiment is referred to below as Exp. 2.

The plants in Exp. 1 were analysed for total nitrogen, soluble sugars and total water-soluble carbohydrate (soluble sugars + starch) on a wet weight basis. In Exp. 2 enough material was available to fractionate the total nitrogen into protein nitrogen and non-protein nitrogen. Fig. 1 shows the relationship between percentage total nitrogen and the number of young produced by three apterae in Exp. 1. It shows clearly that, in general, the higher the percentage total nitrogen the greater is the number of young produced. The correlation coefficient between the two

variables is 0·6741, $P>0{\cdot}01$ but $P<0{\cdot}02$. Since, however, the two sets of plants differ markedly as regards their carbohydrate content, *vide infra*, it is better to consider each set separately. When this is done, the correlation coefficient within the group reared under normal conditions of light is 0·9173, $P=0{\cdot}01$, a value clearly significant, while that within the group reared under subnormal light conditions is not significant, $r=0{\cdot}1486$. In Exp. 2 the correlation coefficient between percentage total nitrogen and the number of young descended from one female is 0·8564; this is highly significant, $P<0{\cdot}01$. In this experiment, it is possible to

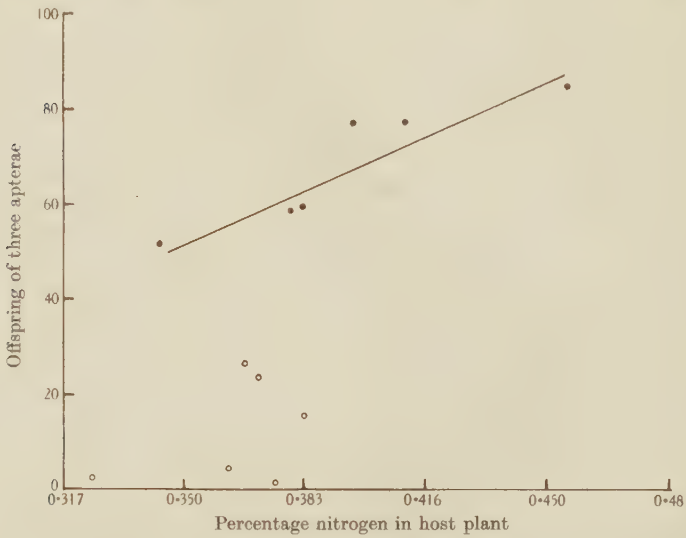

Fig. 1. The relation between reproduction of *Brevicoryne brassicae* and percentage nitrogen in the host plant: Exp. 1. • Plants grown in normal conditions of light. o Plants grown in subnormal conditions of light.

examine the correlation between reproduction and percentage protein nitrogen and percentage non-protein nitrogen, Table II. In the first case the correlation coefficient is 0·8456, $P<0{\cdot}01$, in the second case $=0{\cdot}6776$, $P=0{\cdot}05$. Thus it would appear that the protein content of the plant is the more significant variable. If the two correlations are analysed and the effect of percentage protein nitrogen eliminated, the correlation between reproduction and percentage non-protein nitrogen is reduced to a non-significant level. If the reverse process is performed, the correlation coefficient between reproduction and percentage protein is slightly reduced but still significant, $r=0{\cdot}7577$, $P=0{\cdot}02$. Thus, it is concluded

that the protein content of the plant is the important source of nitrogen for this sucking insect.

Table II

Effect of chemical composition of plant on reproduction

No. of young descended from one female	80	97	113	114	182	243
% Protein N	0·171	0·146	0·184	0·159	0·214	0·248
% Non-protein N	0·064	0·046	0·049	0·052	0·108	0·062
% Soluble sugars	0·093	0·071	0·075	0·089	—	0·137
% Water-soluble carbohydrates	0·262	0·232	0·250	0·237	—	0·445
No. of young calculated when effect of % N is eliminated	189	320	227	283		165
No. of young descended from one female	252	304	337	478	669	760
% Protein N	0·195	0·221	0·287	0·209	0·212	0·339
% Non-protein N	0·052	0·043	0·075	0·067	0·043	0·092
% Soluble sugars	—	0·111	0·235	0·091	—	0·142
% Water-soluble carbohydrates	—	0·303	0·586	0·263	—	0·368
No. of young calculated when effect of % N is eliminated		341	130	485		381

In Exp. 1 the means of the nitrogen contents of the two sets of plants are barely significantly different when examined by means of the t test, $P = 0{\cdot}05$. A consideration of the distribution of the points in Fig. 1 strongly suggests that the much lower rate of reproduction found on plants grown under subnormal conditions of light is due to a factor other than nitrogen content. That this factor is the carbohydrate content of the plant is suggested by Figs. 2 and 3. Since the percentage total nitrogen would account only for a small portion of the difference between the two sets, it has been considered permissible to draw a continuous curve through both sets of data to illustrate the type of relationship obtaining between the variables under consideration. It will be seen that there is a lower limit of carbohydrate content at which reproduction is just possible, three sets of three females producing one, two and four young which died before the experiment terminated. As the carbohydrate content of the plant increases, reproduction increases rapidly until it reaches a maximum and then declines or remains constant. The curve relating reproduction with percentage soluble sugars suggests that an excess of sugar produces a decline in reproduction, but the corresponding curve showing the relationship with percentage total water-soluble carbohydrate suggests that once a maximum is reached an excess of carbohydrate has no adverse effect. Since the percentage total nitrogen plays such a big part in determining the rate of reproduction under normal light conditions, its effect was eliminated statistically and the resultant figures

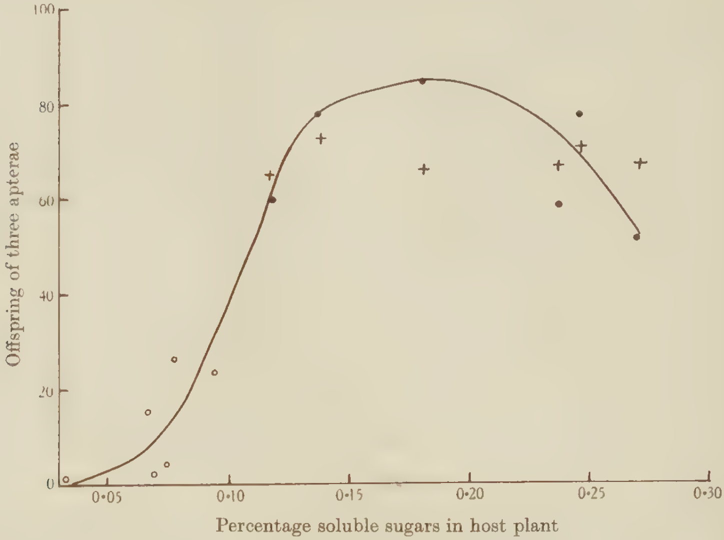

Fig. 2. The relation between reproduction of *Brevicoryne brassicae* and percentage soluble sugars in the host plant: Exp. 1. • Plants grown in normal conditions of light. o Plants grown in subnormal conditions of light. + + for explanation see text.

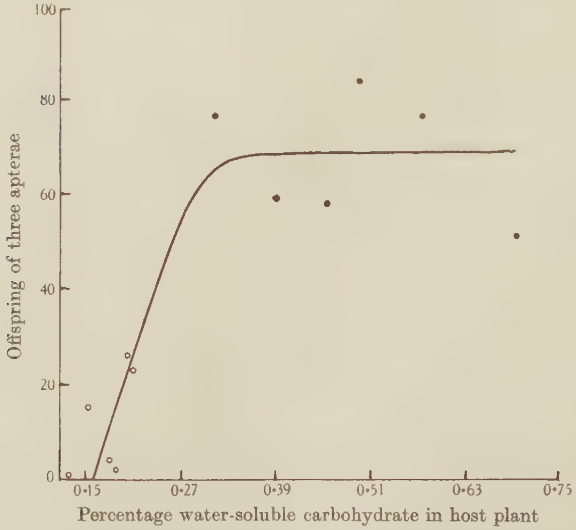

Fig. 3. The relation between reproduction of *Brevicoryne brassicae* and percentage total water-soluble carbohydrate in the host plant: Exp. 1. • Plants grown in normal conditions of light. o Plants grown in subnormal conditions of light.

show that the apparent depressant effect of excess soluble sugars is fortuitous. The resultant figures are indicated in Fig. 2 by crosses. No clear relationship was found between the percentage soluble sugar or percentage total carbohydrate and reproduction in Exp. 2. The correlation coefficient between percentage soluble sugars and reproduction is 0·4070 and is not significant.

The carbohydrate content of the plant might play a critical part in determining the rate of reproduction during the short dull days of early spring and late autumn in some species of aphides. Watson (1936) finds it necessary to illuminate with artificial light for 2 hr. daily the radish and turnip plants on which she rears *Myzus persicae* from November to January in order to keep the insects alive. The aphides are reared in a heated greenhouse, therefore temperature is not a limiting factor. It is possible that, under normal conditions of light at these times of the year, the carbohydrate content of the plant falls below the minimum requirements of the insects and the extra hours of illumination serve to increase it above that minimum. *B. brassicae*, however, reproduces slowly during the winter if the temperature is suitable (Petherbridge & Mellor, 1936).

The present data do not show whether percentage soluble sugars or percentage total water-soluble carbohydrate is the significant variable in the relationship between the aphides and the carbohydrates of the plant. Since there is a highly significant correlation between the percentage soluble sugars and the percentage total water-soluble carbohydrate, a definite decision cannot now be made upon the point. In Exps. 1 and 2 the correlation coefficients between percentage soluble sugars and percentage total water-soluble carbohydrate are 0·9607, $P<0{\cdot}01$ and 0·9794, $P<0{\cdot}01$ respectively. Davidson (1923) has examined sections of plants infested by aphides and finds that they feed upon the contents of the phloem elements in the vascular bundles, but that cells of the epidermis, cortex, mesophyll and xylem may be tapped for nourishment, especially when the host is heavily infested or in poor condition. Thus, whether the soluble sugar content or the total water-soluble carbohydrate is the significant variable may depend on the density of the population of aphides on the plant. When this is low the insects feed principally on the contents of the phloem in which starch is absent and, since the concentration of sugars in the phloem is a function of the concentration of sugars in the leaf, it would appear that the latter would be a valid estimation since it is, at present, impossible to analyse the contents of the phloem.

Since the aphides feed principally on a food which is not known to

contain starch, the presence of a starch hydrolysing enzyme in the salivary glands (Davidson, 1923) may appear somewhat peculiar. Its usefulness may, perhaps, be dependent on circumstances. When the population density on the plant is low, and the aphides are feeding on the phloem contents, its value is nil. When, however, the population density is high and the aphides are feeding on the contents of starch-containing cells, the injection of extra amylase may serve to hasten the hydrolysis of the starch into soluble degradation products.

No data have yet been obtained on the effect of the chemical composition of the host plant on the rate of growth of aphides but Table III gives some data on the weights of larvae of *Pieris brassicae* fed on cabbage leaves grown under the experimental conditions detailed above for Exp. 1. The larvae had a mean weight of 0·2 mg. when the experiment commenced; ten individuals were reared under each of the two conditions of nutrition and were weighed individually at intervals. On and after the tenth day the mean weights of the two groups differ significantly as do those of the sixth day. It is thought that the similar mean weights for the two batches on the eighth day are due to the fact that the larvae grown on normal food were preparing to moult when weighed but the others were still feeding so that the former lot were weighed with empty alimentary canals. The larvae fed on normal food moulted about 12 hr. before the others, ceased to feed 2 days before and pupated 3 days before the others.

Table III

Effect of host plant grown under (A) *normal light conditions,* (B) *subnormal light conditions, on increase in weight in mg. of* Pieris brassicae *larvae*

Days	2	6	8	10	13	17	21	23	25	Pupation
A	0·8	4·4	6·7	29	94	319	618	613		452
B	0·8	3·4	6·5	16	46	161	415	504	562	389
Ratio B : A	1 : 1·0	1 : 1·3	1 : 1	1 : 1·8	1 : 2	1 : 2	1 : 1·5	1 : 1·2		1 : 1·2

The ratio of the weight of the larvae fed on food grown under subnormal light conditions to that of larvae fed on food grown under normal light conditions shows that the latter grew much more rapidly than the former during the first half of the growth period so that they became twice as heavy but, during the last instar (17th day and onwards), the former were able to make up some of the lost ground and, at pupation, weighed but little less. In spite of the great difference in weight of the larvae at the commencement of the last instar, no significant difference was noted in the width of the shed head capsules.

IV. The effect of the insect on the chemical composition of its host plant

A number of small plants were caged under cellophane and infested on 22 August 1936 with varying numbers of apterous aphides. Reproduction was allowed to continue until some of the plants were thoroughly infested. The experiment terminated on 14 September. To obtain an index of the intensity of infestation, the total number of aphides present on the plant was divided by the weight of the leaf laminae in grammes. The index of infestation so obtained is called the population density and, in this experiment, it varied from 109 to 413. Unfortunately, the plants used in this experiment were somewhat varied in size to begin with, and it is thought that the varying chemical composition of the plants associated with varying vigour of growth has obscured the results obtained. This experiment is referred to below as Exp. 3. No significant correlation was found between population density and either plant weight, percentage dry weight, percentage soluble sugars or percentage total carbohydrate. In another set of data (not yet analysed completely) a very definite relationship has been found between population density and the wet and dry yields of leaves and percentage dry weight. There is, however, in Exp. 3, a negative correlation between population density and percentage total nitrogen, $= -0{\cdot}7430$, $P > 0{\cdot}02$ but $P < 0{\cdot}05$. The percentage total nitrogen was analysed into percentage protein nitrogen and percentage non-protein nitrogen but no significant correlations were found. The results, however, suggest that relationships may exist, but discussion of them must be postponed until further data are available.

V. The effect of the chemical composition of the host plant on the production of alatae

Various explanations have been put forward to account for the appearance of winged aphides under different conditions. It has been established that a higher proportion of alatae occurs on over-crowded plants than on sparsely infested plants (Reinhardt, 1927). Shinji (1918) produced some evidence that solutions of various salts and organic substances, especially sugar, when introduced into infested rose cuttings would cause variations in the percentage of alatae ultimately produced on the shoots. However, confirmatory evidence was not obtained by several later workers. Ackerman (1926), Reinhardt (1927), and Wadley (1931) found that starvation of either adults or first or second instar nymphs, according to the species of aphis, would induce the formation of

alatae. Shull (1930) studied this problem in great detail with reference to light, attempting at the same time to eliminate any effect of the chemical composition of the plant on the formation of alatae. He claimed that he was able to demonstrate that varying times of exposure to light and varying intensities of light caused a variation in the percentage of alatae produced, and that the effect was directly due to light. However, he was forced to conclude in one experiment that it was probably the chemical composition of the plants which caused the production of alatae. Shull did not analyse his plants and so his claim that the chemical composition of the plant did not affect his results must be regarded with caution until his elaborate experiments are repeated together with analyses of the plants. Rivnay (1937) has recently produced evidence to show that, in *Toxoptera aurantii* Boy., lack of water in the shoot on which the insects are feeding may bring about the production of alatae, but his sweeping assertions that "temperature does not affect wing development in Aphids" and that "all such factors as light, temperature, crowding, humidity, precipitation, growth of plant, etc., exert, directly or indirectly, an influence on the water balance in the body of the aphid which in turn causes wing development" cannot be admitted until much more work is done on more than one species of aphis. Most writers are agreed that it is unsuitable conditions in the host plants which bring about the formation of alatae and so it is quite possible that, according to circumstances, different factors within the host plant may have this effect. Evidence will be adduced below to show that in *B. brassicae* the protein content of the host plant had very definite influence on the production of alatae.

In Exp. 3 a varying percentage of alatae occurred on the different plants; the correlation coefficient between population density and percentage alatae was not quite significant, $r = 0{\cdot}6697$, $P > 0{\cdot}05$ but $P < 0{\cdot}10$, but the correlation coefficient between percentage total nitrogen and percentage alatae was much higher $= -0{\cdot}8199$, $P > 0{\cdot}01$ but $P < 0{\cdot}02$. As figures are available for the percentage protein nitrogen and percentage non-protein nitrogen, it is possible to examine which of these fractions is the more significant in determining the production of alatae. The correlation coefficient between percentage alatae and percentage protein nitrogen is $-0{\cdot}8825$, $P < 0{\cdot}01$, while that between percentage alatae and percentage non-protein nitrogen is $0{\cdot}6266$, $P = 0{\cdot}10$. There is, therefore, a highly significant negative correlation between the production of alatae and the protein content of the plant, but the correlation between percentage alatae and percentage non-protein nitrogen is not

significant. Fig. 4 shows that in Exp. 2 there is clearly a relationship between percentage alatae and both percentage total nitrogen and percentage protein nitrogen. No relationship is to be found between percentage alatae and percentage non-protein nitrogen in this experiment. Table IV gives the data obtained from Exp. 2 on this point.

With regard to the relationship between percentage alatae and the carbohydrate content of the plant, none was found in Exp. 3 but in Exp. 2, Table IV shows that there is an increasing percentage of alatae as the amount of soluble sugars and of total water-soluble carbohydrate diminishes. In this experiment, it is not possible to differentiate between

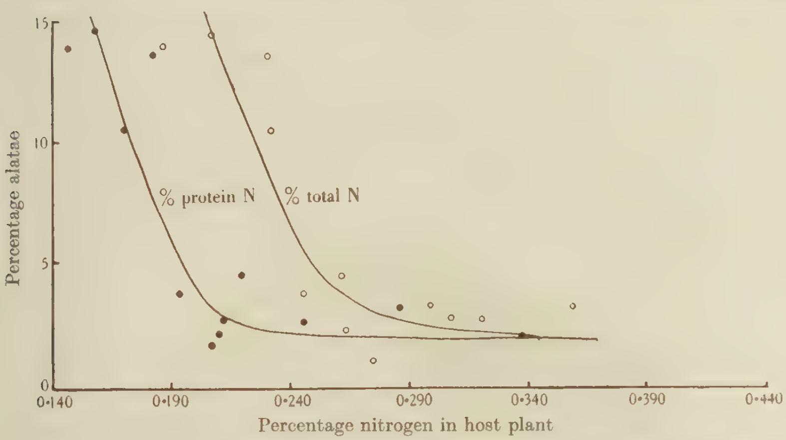

Fig. 4. The relation between the formation of winged forms of *Brevicoryne brassicae* and the percentage of nitrogen in the host plant: Exp. 2.

Table IV

The effect of the chemical composition of the host plant on the production of alatae

Exp. 2.

% Alatae	14·8	14·3	13·9	10·7	4·8	4·0	3·4	2·9	2·2	2·1
% Protein N	0·159	0·146	0·184	0·171	0·221	0·195	0·287	0·248	0·339	0·209
% Non-protein N	0·052	0·042	0·049	0·064	0·043	0·052	0·075	0·062	0·092	0·067
% Soluble sugars	0·089	0·071	0·075	0·093	0·111	—	0·235	0·137	0·142	0·091
% Total water-soluble carbohydrates	0·237	0·232	0·250	0·262	0·303	—	0·586	0·445	0·368	0·263
% Water	92·5	93·0	92·1	92·4	92·4	92·9	89·4	91·0	90·7	92·1

Exp. 3.

% Alatae	26·1	23·4	22·9	18·5	12·0	9·1	3·4	1·6
% Total N	0·304	0·285	0·283	0·293	0·310	0·335	0·333	4·14
% Protein	0·173	0·166	0·194	0·211	0·209	0·250	0·254	0·336
% Water	90·9	92·1	91·5	92·3	91·3	92·0	91·8	88·6

Table V

The effect of carbohydrate on the production of alatae

Treatment...	Light					Dark					Very dark				
Cage ...	1	2	3	4	Mean	1	2	3	4	Mean	1	2	3	4	Mean
% Alatae	2·9	7·4	4·0	4·1	4·6	2·5	6·1	4·0	4·5	4·3	0	3·2	0	9·3	3·1
% Soluble sugars	0·243	0·261	0·192	0·257	0·238	0·221	0·189	0·238	0·177	0·206	0·092	0·075	0·077	0·149	0·098
% Total water-soluble carbohydrates	0·632	0·719	0·454	0·611	0·604	0·537	0·492	0·571	0·418	0·505	0·247	0·151	0·233	0·337	0·242
Water	87·1	90·8	90·7	86·5	88·8	92·9	93·5	92·0	92·0	92·6	93·2	93·6	94·1	93·1	93·1

the effect of protein and carbohydrate on the production of alatae. An experiment was set up to test whether the carbohydrate content of the plant had any effect on the production of alatae. A set of twelve cabbage plants, selected for uniformity of size, were divided into four blocks of three plants and subjected to the following treatments of light intensity, (*a*) cellophane cages, (*b*) cellophane cages covered with one layer of black voile, (*c*) cellophane cages covered with two layers of black voile. The results are given in Table V and show conclusively that wide variations in the carbohydrate content of the plant do not affect the production of alatae.

Tables IV and V also show that the percentage of water in the plants had no effect on the production of winged forms in *B. brassicae*. It is, of course, possible that the critical factor in the plant that brings about wing formation will vary from species to species according to the host plant and climatic conditions; thus in the xerophytic conditions under which *Toxoptera aurantii* lives, water might well be a critical factor. The problem as to what factors within the aphis induce the production of wings is as yet unstudied except for the suggestion of Rivnay that a low water content will bring it about and some speculations by Ackerman (1926) and Shull (1930) who postulate the presence of a substance which by concentration or change into another substance respectively determined whether apterae or alatae will develop. A study of the relation between ductless glands, such as the corpus allatum, and wing production might produce some interesting results.

No data on the effect of the quality of the food on the rate of feeding have been obtained and the work of Hamilton (1935) shows that to obtain any accurate data of this kind would be very difficult. Some information bearing on this factor has, however, been obtained with larva of *Pieris brassicae*. Half-grown larvae were fed on normal cabbage leaves

and on leaves kept under a double thickness of black voile for 24 hr. The weights of faeces voided by each of five larvae feeding on the two types of leaves for 18 hr. was recorded. From the normal leaves an average of 0·081 g. was produced; from those kept under dark conditions an average of 0·171 g. The mean increments in growth of the batches of larvae during the experiment were very similar, being 37 and 39%, so that the difference in the mean weights of the faeces is not due to the larvae which had fed on normal food utilizing more of it than those fed on leaves kept under dark conditions. The conclusion drawn from the data is that the larvae feeding on the food of poor quality (especially in carbohydrates) actually consumed much more than those feeding on normal leaves.

When the percentage protein nitrogen in the plant reaches a low level, there is a rapid increase in the percentage alatae of *Brevicoryne brassicae* produced. Whether this increase is due to the quantity of nitrogenous food falling below a definite required amount necessary to produce apterae or to a change in the quality of the protein is not known. It is hoped that this aspect of the problem will be studied later.

VI. SUMMARY

It is shown that under late summer conditions of light the rate of reproduction of the aphis, *Brevicoryne brassicae*, is positively correlated with the nitrogen content of the host plant and, in particular, with the protein content. The formation of winged forms is negatively correlated with the same factors.

The chemical composition of the plant affects the rate of growth, length of larval period and final pupal weights of *Pieris brassicae*. It also influences the amount of food eaten.

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SHEEP BLOW-FLY INVESTIGATIONS

VII. OBSERVATIONS ON THE DEVELOPMENT OF EGGS AND OVIPOSITION IN THE SHEEP BLOW-FLY, *LUCILIA SERICATA* MG.

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Introduction

Although *Lucilia sericata* Mg., the sheep maggot-fly, has been the object of intensive study by insect physiologists during recent years, comparatively little attention has been paid to the adult stage. This is surprising, since it is the adult female's habit of ovipositing on live sheep that is primarily responsible for the enormous damage caused by this species. Accordingly, investigations have been carried out on blow-flies with reference to the development of eggs and oviposition. The previous work need not be reviewed here in detail since Evans (1936) has recently summarized our knowledge of the physiology of *L. sericata*. Whereas the males become sexually mature within a few hours after emergence, the females require several days before the ovaries are fully developed; furthermore, this only occurs if the diet includes meat. On the other hand, fertilization by the male is not necessary for the development of the ovaries or for oviposition; my experience has been, however, that unfertilized females do not oviposit readily. When a female oviposits, the ovaries are as a rule completely emptied of eggs, and a fresh batch of eggs develops later. The number of eggs in a batch depends on the size of the fly and varies from about 80 to 170.

Experimental

Effect of temperature on the development of the ovaries

For these investigations the flies were kept in jam jars half-filled with sand and placed in incubators; to prevent the flies escaping, their wings were clipped and the jars were covered with gauze. The flies were fed on sugar, meat and water, which were renewed daily; the humidity was kept high by the drinking water in the jars and by water exposed in the incubator. Provided that only a small piece of meat was placed in the

jar, the flies remained healthy[1] under these conditions, and, if fertilized, oviposited at regular intervals. For studying the initial development of the ovaries, female flies were taken as soon as they emerged from the pupal cases and transferred to jars in batches of about eight. The growth of the ovaries was followed by dissecting flies at intervals. In order to investigate the development of eggs subsequent to the first oviposition, it was necessary to use fertilized females, and the following procedure was adopted. A number of recently emerged flies were placed in a cage and fed on meat, sugar and water until oviposition began; the meat was then taken away and at intervals females were removed and placed separately in test-tubes containing meat. Those that oviposited were then transferred to incubators; each fly was kept in a separate jar and the times of oviposition were noted.

Table I

Effect of temperature on the initial development of the ovaries in recently emerged females

Temperature (° C.)	Days required for development of mature eggs
37	2
30	3
23	4·5
19·5	7
15–16	13

Table II

Effect of temperature on the production of eggs by fertilized females

Temperature (° C.)	Interval in days between 1st and subsequent ovipositions	
	Limits	Average value
37	1·0–1·5	1·2
30	1·0–1·7	1·4
23	2·0–3·0	2·4
19·5	2·9–3·8	3·3
15–16	4·5–8·0	5·5

Table I shows the effect of temperature on the development of the ovaries in recently emerged females. These values are in good agreement with those obtained by Mackerras (1933) and Evans (1935) with this species. Mackerras found mature eggs after 5–6 days at 20–22° C., Evans after 6 days at 23° C. Table II shows the results obtained with fertilized

[1] In an experiment with flies fed on dung in jars, one of the flies died from an infection with *Empusa* species. At about the same time (November 1937) all the flies in one of the cages died from the same cause.

females which had oviposited once. It will be seen that, at corresponding temperatures, the second and subsequent batches of eggs develop in about half the time required by the first batch. With regard to the extreme temperatures used, there was evidence that these were unfavourable. At 37° C. mortality was high, and at 15–16° C. oviposition was irregular, the interval increasing considerably after the third oviposition. The figures shown in Table II for this temperature refer only to the first three ovipositions. At other temperatures, the intervals were regular except that they tended to decrease slightly after the second oviposition. It should be noted that these figures represent minimum values since the flies were kept under optimum conditions of nutrition. In nature, the development of the ovaries will be slower as the fly has to search for food.

Effect of nutrition on the development of the ovaries

Although previous workers have shown that a meat diet is essential if the eggs are to develop in the ovaries, no critical examination appears to have been made on the minimum number of meat meals necessary. In order to investigate this point, some recently emerged (unfed) females were divided into three groups and kept in jam jars at 23° C., sugar and water forming the basal diet. Meat was fed by placing the flies separately for 4 hr. in test tubes containing liver; generally, the fly had finished feeding after 2–3 hr. The first group received one feed of liver on the second day, the second group two feeds on the second and fifth days, the third group three feeds on the second, fourth and sixth days. On the eighth day all the flies were killed and dissected. It was found that the ovaries were only partly developed in the flies receiving one meal of liver; mature eggs were present in flies which had been given two or three meals. When beef muscle was substituted for liver, mature eggs were found in flies receiving three meals but only in some of the flies receiving two meals. This difference was probably due to the fact that flies cannot extract so much juice from muscle (butcher's meat) as from liver; this was shown to be so by weighing flies before and after feeding on muscle and liver. Clearly, at least *two* meat meals are necessary for the development of mature eggs in recently emerged flies. Similar experiments were carried out with fertilized females that had oviposited once. These showed that two meat meals are necessary, also, for the regrowth of eggs after oviposition; this includes the meal taken when laying; i.e. if a gravid female was allowed to feed and oviposit on liver, mature eggs were not found later unless at least one meat meal was given in the interim.

In these experiments the flies were always kept for at least 6 days at 23° C. after the first meat meal, this period being adequate for egg development on a meat diet (Tables I and II). Yet in all cases where immature eggs were found owing to insufficient meat, the eggs had reached the same stage of development. Under normal conditions, the eggs first appear in the ovaries as small spheres; later they become oval, then elongate further and become opaque. In flies which had received only one meat meal, the eggs were always found to be in the small spherical stage and never partly elongated. This indicates that the process occurs in two stages, and that the second stage, which includes elongation, does not begin until the fly has obtained sufficient meat for the eggs to become mature.

In order to find out how much juice a fly takes when feeding on liver, females were starved overnight and then weighed before and after feeding. It was found that a fly may consume as much as 15 mg. of juice. Since two meals are required for the development of a batch of eggs, not more than 30 mg. of liver juice are necessary in addition to carbohydrate.

Feeding experiments were also carried out to determine whether meat can be replaced by other substances. These tests were carried out in jam jars, as before, the flies being fed for a week on sugar, water and the test substance, and then dissected and examined. Experiments were made both with recently emerged flies and ones which had already oviposited. In some cases tests were carried out in a similar way in cages. The results are shown in Table III.

Table III

The effect of different foods on the development of the ovaries

Substance added to basal diet of sugar and water	Development of ovaries
Meat	+
Blood serum	–
Blood serum + marmite	+
Milk	–
Sheep dung	–
Dog dung	–
Pollen	–
Ivy flowers	–
Blackberries	–

These findings are in agreement with those of previous workers; thus, Mackerras (1933) found no development of the ovaries in blow-flies fed on various plant products or sheep dung. In the present experiments faeces from a carnivorous animal were included, but these also gave

negative results. It will be seen that none of the plant materials tested can replace carrion. The pollen used was obtained from pollen cells in beehives. Ivy flowers were tested as it was noticed that these attract blow-flies in considerable numbers. The results with serum suggest that the food requirements of the female blow-fly include salts and vitamin B in addition to protein. Blood serum is rich in protein of good quality, but deficient in potash, phosphate and vitamin B (Hobson, 1935*a*). Marmite supplies these substances, and Table III shows that eggs develop in flies fed on serum if this is supplemented with marmite. Mackerras (1933) has shown that a *L. sericata* female may produce as many as 2000 eggs, i.e. at least four times its own weight. Clearly, all the necessary constituents must come from the food. When carbohydrate is supplied in the form of pure cane sugar, as in these experiments, the meat must supply salts and vitamins in addition to protein. Under natural conditions this may not be so, since the fly obtains its sugar from plant juices which contain also water-soluble vitamins and salts. The failure of flies to produce eggs when fed on milk is interesting since this is usually regarded as a complete foodstuff; this result suggests that blow-flies may require specific accessory factors present in meat.

Chemotropic responses of gravid females

This subject is of great fundamental importance since the attraction of sheep for *L. sericata* is the initial cause of maggot infestation. Observations in the field have shown that the attraction is twofold; one factor is supplied by the sheep and the other by putrefying material or certain products of putrefaction, such as ammonium carbonate, indole or skatole (Hobson, 1935*b*; 1936). Furthermore, this attraction is specific for gravid females of *L. sericata*; it is therefore necessary to use gravid females for studying this response in the laboratory. The method used for obtaining gravid females was essentially the same as that described by Evans (1935). Recently emerged flies were fed on sugar and water for 2–3 days; meat was then supplied until the first oviposition occurred, when the meat was removed and the flies fed only with sugar and water. Examination showed that most of the females contained ripe eggs after this treatment. However, the readiness of flies to oviposit varied greatly in different cultures. It was always increased by leaving the flies for several days after removing the meat; also flies oviposited more readily in the summer, probably because they feed better in the presence of sunlight. As a rule, the experiments on oviposition were carried out during the second week after the end of meat feeding.

If gravid females are kept without meat, they occasionally lay on the sides of the cage, on wet cotton-wool, or on sugar, particularly if this has started to ferment. Various substances were, therefore, exposed in the cages to attract oviposition. The earlier experiments gave negative results, but later, with cultures bred during the summer, certain putrefactive products were found to attract females strongly and to stimulate oviposition. This response, however, was very uncertain, and it was found that oviposition could be induced far more readily by placing females in tubes containing the test substances. Liquids were absorbed on cotton wool, which was also used to plug the tubes; the flies were placed in an incubator at 23° C. for several hours, the tubes then being examined for the presence of eggs. Table IV gives a list of substances which stimulated oviposition.

Table IV

List of substances found to stimulate oviposition by L. sericata

Indole	Ammonium carbonate
Skatole	Ethyl alcohol
Ammonia	Suint
Trimethylamine	

In addition, sheep's wool moistened with water occasionally provoked oviposition; however, in control tests with damp cotton wool oviposition also occurred with some cultures. The proportion of flies found to oviposit on these substances was small, about 1 in 10; with the substances tested in Table IV usually four to eight out of ten flies oviposited. The sample of suint used was a water extract of wool which had stood for several months in the laboratory and had decomposed. Oviposition was sometimes found with suint, indole and trimethylamine solutions when exposed in cages, but none of these substances attracted oviposition when exposed in the field. It was found that gravid females readily oviposited on sheep. If the flies were released near sheep, they immediately flew away; but if their wings were cut and they were placed in the fleece, near a pad of cotton-wool soaked in indole solution, they usually oviposited within half an hour. Oviposition experiments were also carried out with wild flies (*L. sericata*) trapped by means of sheep treated with a solution of indole. These flies oviposited on indole or suint, and responded more readily than bred flies. In fact difficulty was experienced in transporting the flies to the laboratory as many oviposited in the tubes before they had been tested.

These observations show that the oviposition response consists of two distinct phases: (1) attraction from a distance, and (2) stimulation to oviposit. The first depends upon two factors in the case of sheep, supplied by the live animal and by products of protein decomposition (Hobson, 1935*b*, 1936). Similarly, the attraction of carrion may consist of putrefactive products combined with other odoriferous substances present in carrion. The second phase, oviposition, depends upon several factors; although it can be produced by certain chemicals, it is also a tactile response. A meal often seems to stimulate oviposition, probably because it distends the abdomen and the ovipositor is then more liable to touch the object. For this reason it is usually easier to provoke oviposition on meat than on other materials which do not supply food.

Effect of nutrition on oviposition

Experiments on oviposition in *L. sericata* are rendered difficult by the variation in response even among flies bred in the same cage under identical conditions. When the flies were kept in the dark in jars, as in the temperature experiments, oviposition occurred at regular intervals. Light, therefore, has no direct effect, but under these confined conditions, being very close to the food, the flies were always gorged with food. When kept in cages, flies are more active and feed more freely at high temperatures and in bright light. Differences in nutrition were probably the cause of the variation in oviposition responses and the better results obtained in summer. Direct evidence for this was obtained from experiments with fertilized flies, which, after laying on liver, were kept in jars and fed with liver every second day. Oviposition occurred at the third meal in some cases, i.e. when two meals had been digested since the previous oviposition. However, eggs were only laid in response to indole by flies that had received three meat meals since the last oviposition. This suggests that a diet rich in meat is necessary before a female will lay on substances other than carrion. This was shown also by feeding gravid females on meat for a short time and testing them the next day with indole; the meat-fed flies laid more readily than females from the same cage which had not received meat.

Discussion

The results of the nutrition experiments with blow-flies indicate that carrion plays an important part in the infestation of sheep with maggots. Carrion seems to be an essential food for the development of the ovaries in the female, since it cannot be replaced either by animal excreta or

plant materials. Theoretically, then, fly attack might be controlled by the destruction of all carrion, but the following considerations will show that this means of control is impracticable, apart from the question of cost. *L. sericata* has a flying range of at least 4 miles according to Gurney & Woodhill (1926), and a very small animal, such as a dead bird or mouse, would supply innumerable flies with sufficient food for the development of the ovaries. It is of interest, in this connexion, to calculate the amount of food required by the female fly for the development of ripe eggs and by the larvae emerging from these eggs. The fly requires two meals, i.e. about 30 mg. of meat juice. Salt (1932) has shown that each larva requires at least 150 mg. of meat for full development. If the fly lays 100 eggs, the emerging larvae will require 15,000 mg., apart from competition from larvae of other blow-flies. It is clear that a scarcity of carrion will react far more on the larval stage than on the fecundity of the adults. However, carrion is probably not a limiting factor in the case of *L. sericata* larvae since live sheep are an alternative breeding place; larvae growing on sheep do not suffer from competition with other species or from the attack of parasites (Davies, 1930).

Although, owing to the abundance of small dead animals, complete destruction of carrion is impracticable, attention should be paid to the more important sources of carrion, so as to prevent the population of gravid blow-flies becoming excessive. These sources include dead farm animals, rabbit warrens, meat exposed in shops or in carts during transit. Macleod (1937) has recently suggested trapping by the old-fashioned method of hanging carrion over water, into which the maggots eventually drop and are drowned. Although this method traps the eggs, it may do more harm than good since it supplies the adults with food without catching them.

With regard to the oviposition experiments, an interesting point is the difficulty of rearing gravid females which are ready to oviposit. A diet rich in meat is essential if flies are to oviposit on some object other than carrion, but meat must not be kept too long in the cages, otherwise the females oviposit and are no longer gravid. Yet in the field during the summer gravid females are quickly attracted in large numbers to a sheep infested with maggots or treated with indole. Before a fly can oviposit on a sheep, it must have at least two, perhaps three, meals of carrion. Why, if it can find carrion for feeding purposes, does the fly not lay its eggs on carrion? There seems to be three possible explanations: (1) Carrion is a limiting factor, and blow-flies have difficulty in finding carrion for feeding and oviposition. Females (*L. sericata*) which become gravid

are, therefore, liable to be attracted to sheep owing to lack of carrion. (2) Carrion is abundant and the females are continually becoming gravid, ovipositing and becoming gravid again. Owing to the high population of *L. sericata* and their keen scent, gravid females soon find their way to a sheep that has become attractive. If a gravid female is not quickly attracted to sheep, it may oviposit on carrion, but it soon becomes gravid again owing to abundance of food. (3) *L. sericata* is attracted to carrion only for feeding purposes and not to oviposit.

The first explanation seems unlikely since the amount of carrion required by the adult for feeding and as a focus for oviposition, is small compared with that needed by the larva. Only an abundance of carrion, leading to the production of large numbers of flies, followed later by a dearth, would make carrion a limiting factor of adult fecundity. The second and third explanations are, therefore, more likely; also, they are not incompatible. The relative attractiveness of sheep and carrion for gravid females is clearly an important factor. This might be tested by examining the proportion of gravid and non-gravid females visiting carrion, comparison being made between *L. sericata* and other species which breed only on carrion; or by determining what proportion *L. sericata* forms of the total catch in meat traps of (1) eggs, (2) adults. It seems possible that females of this species may frequent districts rich in carrion when they are not gravid, migrating to sheep grazings when gravid. Thus, it has been shown that the attraction supplied by sheep is specific for gravid females (Hobson, 1936). Also, Morison (1937) found that *L. sericata* formed only 3 % of the blow-flies caught in meat traps and he obtained evidence that this species is more abundant in the proximity of sheep.

Although *L. sericata* readily oviposit on carrion under cage conditions, it does not necessarily follow that carrion is highly attractive to gravid females in the field. The present experiments have shown that the oviposition response normally consists of two distinct phases, attraction from a distance and stimulation to oviposit. Indole, for example, stimulates gravid females to oviposit, but does not attract them (except in conjunction with live sheep). The oviposition experiments have shown that various putrefactive products stimulate oviposition; little progress, however, has been made in finding substances which will attract gravid females (without live sheep) in the field.

Summary

1. An investigation has been made of the effect of temperature upon the time required for the development of the ovaries in the sheep maggot fly.

2. It has been shown that two meat meals are essential for the development of mature eggs in the ovaries. This applies also to the further production of eggs after oviposition.

3. Neither plant materials nor animal excreta can replace meat in the diet of the female fly if eggs are to develop in the ovaries.

4. Various products of putrefaction stimulate gravid females to oviposit, but these substances do not attract flies from a distance.

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SOME APPLICATIONS OF LABORATORY BIOLOGICAL TESTS TO THE EVALUATION OF FUNGICIDES

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CONTENTS

Introductory

In a recent paper (Horsfall *et al.* 1937) the factors determining the field performance of a fungicide are separated into two groups. Included in the first group are the quantity factors governed by physical and chemical properties which determine the retention and tenacity of the spray deposit and, thus, fix the amount and evenness of distribution of the material on the substratum. The second group constitutes the quality factors making up fungicidal value. Fungicidal value is defined as the resultant of (1) the ease of formation of an active fungicide from the spray deposit (i.e. availability), and (2) the relative inherent toxicity of the active material thus produced. Recent work by McCallan & Wilcoxon (1936) has suggested a relation of availability to the presence

of solubilizing spore excretions. For the present, however, fungicidal value cannot be assessed on purely chemical data, and a biological technique is required for the double purpose of rendering the fungicide available and registering the toxicity of the compound produced.

The use of fungus spores in such biological tests is a generally accepted procedure which has the advantage of rapidly giving results that can be simply expressed and readily compared. When the aim is the evaluation of materials as protectant fungicides, the approved method is that of sowing spores in contact with the dried residue of a fungicide applied by a standardized spraying technique (McCallan, 1930).

In the present investigation, this method has been used for a sorting out, in terms of relative protectant value, of a number of sulphur and copper compounds, for many of which fungicidal properties had been claimed. Further, this technique has been employed for giving a biological demonstration, paralleling the chemical proof, of the *in vitro* effect of certain spray supplements on the tenacity of fungicidal spray deposits.

In considering, however, the absolute concentration of a fungicide (with or without supplement) necessary to give control of a specific disease in the field, the applicability of results from *in vitro* tests remains in question (Montgomery & Moore, 1938). A laboratory method of testing fungicides on living leaves has been suggested in a previous paper (Marsh, 1936), and the results of a series of such tests are given below. A limited number of experiments have also been made to determine the amount of agreement between the results of the laboratory leaf tests and those of field trials using the same organisms and materials.

Methods

In carrying out the laboratory tests the materials for examination, after dilution to the required strength, are kept agitated by a mechanical stirrer and sprayed on to cellulosed slides by means of an atomizer operating under standard conditions. The apparatus is fully described by Evans & Martin (1935) and its employment in fungicide tests by Marsh (1936). Spraying is commonly carried out for either 5 or 10 sec. at a pressure of 2 atm., the cellulosed slide being held 2 ft. from the jet. The deposition for a 10 sec. exposure is at the rate of 0·008 ml. spray per square centimetre.

The sprayed slides are dried at laboratory temperature and maintained exposed to the air but protected from dust for at least 24 hr. If

the tenacity of the spray deposit is to be tested, the slides are then held 10 in. from the atomizer jet for 60 sec., and subjected to a distilled water spray at $1\frac{1}{2}$ atm. pressure. After this leaching process the slides are again allowed to dry before being used for a spore germination test.

In carrying out a test the slides are placed on glass racks in moist chambers (McCallan, 1930). On to each slide is then pipetted three separate drops of spores suspended in water. Each drop is approximately 0·04 ml. and contains 150–200 spores; its area of spread on the sprayed slide is a circle of about 7 mm. diameter. In this investigation the spores most frequently employed have been conidia of the Pear Scab fungus (*Venturia pirina*) obtained from young naturally-occurring leaf infections on the variety Williams' Bon Chrétien. Other spores used have been conidia of *V. inaequalis* (from leaves of Crimson Cox apple) and ascospores of *Nectria galligena* (from natural infections on Lane's Prince Albert wood). The sown slides were incubated at 21° C. and the percentage of germinated spores was determined by counting after 48 hr. Each determination was made on at least 600 spores distributed in six drops on a pair of slides.

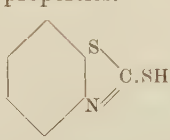
Tests of certain of the materials were also made on individual leaves in the laboratory using the technique described elsewhere (Marsh, 1936). Young leaves of Crimson Cox apple were used for the tests with *Venturia inaequalis* and those of Williams' pear for tests with *V. pirina* spores.

Materials

(i) *Rubber accelerators and allied compounds*

In vulcanizing rubber by the addition of sulphur it is the practice to expedite the reaction by the employment of certain thiazole, thiuram and guanidine derivatives which are referred to under the term of rubber accelerators. These complex organic compounds are accordingly available in commerce and, for certain of them, fungicidal and/or insecticidal properties have been claimed. Further, they are of interest in connexion with the mechanism of the fungicidal action of sulphur. The materials tested are listed below with notes of any previous references to their fungicidal and insecticidal properties.

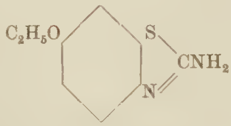
(1) *Mercaptobenzthiazole*:



Patent specifications claim that this material is a disinfectant for seeds, corms and tubers, and that a 0·1% solution of the sodium salt

inhibits the growth of cultures of *Botrytis cinerea*, *Phoma pomi*, *Glomerella cingulata*, *Sclerotinia cinerea* and *Fomes annosus*. Insecticidal properties are also claimed (see Roark & Busbey, 1935). Montgomery & Moore (1938) found that mercaptobenzthiazole was fungicidal to *Venturia inaequalis* spores at 0·01 %.

(2) 6-*ethoxy-2-aminobenzthiazole*:

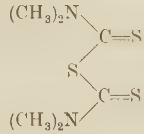


The fungicidal and insecticidal claims made for a 0·1 % solution of the hydrochloride of this compound are similar to those made for mercaptobenzthiazole.

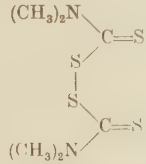
(3) *Dibenzthiazyl disulphide*:

No records of previous fungicidal tests with this material have been found.

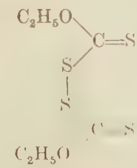
(4) *Tetramethyl thiuram monosulphide*:



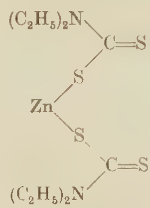
This material has been patented as a seed disinfectant and as a timber and textile preservative to be used at 0·2 %. It is also specified as an insecticide (see Roark & Busbey, 1935). Tests made at Newark, Delaware (Guy, 1937), show that it acts as a repellent to leaf-eating insects. In field tests against Apple Scab at East Malling (Montgomery *et al.* 1935) it was used at 0·07 %, at which concentration it was inferior in fungicidal power to lime sulphur at standard strength. In laboratory tests (Montgomery & Moore, 1938) against Apple Scab spores it was found to be fungicidal at 0·005 %.

(5) *Tetramethyl thiuram disulphide*: 

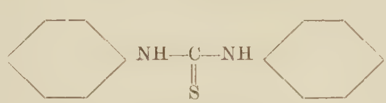
A saturated aqueous solution of this material is said to act as a wood preservative. Added to an agar medium at the rate of 1 part in 250 it inhibited the growth of *Aspergillus niger* and *Fomes annosus* (see Roark & Busbey, 1935). In a field trial at East Malling it was shown to be non-phytocidal on apples at 0·25% (Moore *et al.* 1936). At Newark, Delaware, it is recorded as a repellent (Guy, 1937). Montgomery & Moore (1938) found that it was fungicidal to *Venturia inaequalis* spores at 0·0005%.

(6) *Xanthic disulphide* (synonym—Ethyl dixanthogen): 

This material is not in use as a rubber accelerator but was included in the tests as a compound closely related in structure to the foregoing. Montgomery & Moore (1938) found that the methyl homologue was not fungicidal to *Venturia inaequalis* spores at 0·1%.

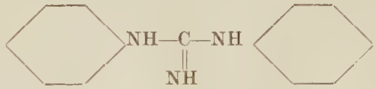
(7) *Zinc diethyl dithiocarbamate*: 

No record of fungicidal tests with this material has been found.

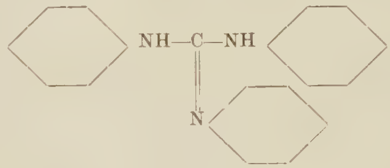
(8) *Thiocarbanilide*: 

This material is stated to be poisonous to leaf-eating insects; it has been suggested for use as a dust on fruit trees in the proportion of 15 parts of thiocarbanilide to 85 parts of sulphur. It is also claimed that a dust containing 3–10% thiocarbanilide may be used on wheat plants to control *Puccinia graminis* (see Roark & Busbey, 1935).

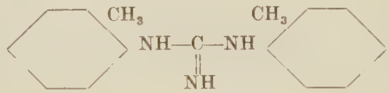
(9) *Diphenyl guanidine*:



(10) *Triphenyl guanidine*:



(11) *Diorthotolyl guanidine*:



No reference has been found to the fungicidal use of these materials. The related compounds, benzthiazole guanidine and benzoxazole guanidine were tested by Wilcoxon & McCallan (1935) who record that they are not promising fungicides.

Materials 1–11 listed above were supplied by Messrs Imperial Chemical Industries, Ltd., in the form of finely divided powders. As these materials are insoluble or very slightly soluble in water they were diluted for testing by dissolving in a minimum quantity of acetone and adding the solution to water containing methyl cellulose as a dispersing agent. The solvent is removed by evaporation on the drying of the spray deposit.

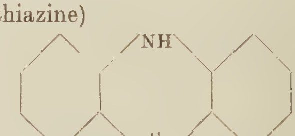
(ii) *Thiocyanates and thiodiphenylamine*

(12) *Lauryl thiocyanate*: $C_{12}H_{25}.SCN$.

This material is in commercial use as an ovicide and acaricide. No reference to fungicidal tests has been found.

(13) *Cetyl thiocyanate*: $C_{16}H_{33}.SCN$.

(14) *Thiodiphenylamine*: (synonym—Phenothiazine)



This material is of importance in dyestuffs manufacture as the parent substance of a series of dyes including methylene blue. As an insecticide it is highly specific but is considered one of the most important synthetic organic compounds for possible use as a substitute for arsenicals. Tested

in dust form as a fungicide it was found non-toxic to spores of *Sclerotinia fructicola*, *Botrytis paeoniae* and *Pestalotia stellata* (Wilcoxon & McCallan, 1935). Against spores of *Venturia inaequalis* it is recorded as effective at 0·1% (Montgomery & Moore, 1938).

The thiocyanates were received in a petroleum oil emulsion. This mixture was not found satisfactory for testing as the emulsion itself showed toxic properties. Further tests were made using the thiocyanates in a sulphite lye emulsion.

Commercial thiodiphenylamine was obtained from three different sources, and a sample of pure steam-distilled thiodiphenylamine was also employed. These materials were diluted for testing either by solution in acetone as previously described, or by grinding up the solid with sulphite lye syrup, and then diluting with water to form a suspension.

(iii) *Copper compounds*

(15) *Cuprous cyanide.*

This material has been successfully employed as an insect repellent but has not found commercial application because of potential hazards to human health. Records of fungicidal tests against spores of *Venturia inaequalis* are given in a previous paper (Marsh, 1936). Phytocidal effects on apple are recorded by Montgomery *et al.* (1935) and by Marsh *et al.* (1937).

(16) *Cupric ammonium silicate.*

Experiments in New Jersey have shown that a range of copper ammonium silicates can be prepared having differing copper availabilities. From this range a material was selected by Sessions (1936) which gave excellent control of apple scab in a field trial.

(17) *Cuprous thiocyanate.*

(18) *Cupric oxalate.*

(19) *Cupric phthalocyanine.*

The last named has been employed as a pigment: no insecticidal or fungicidal properties have been claimed for this or for the two preceding compounds.

In the present investigation, Nos. 15–19 were obtained as paste suspensions from Messrs Imperial Chemical Industries, Ltd. For testing they were diluted with water to give concentrations of 0·1% copper and 0·2% copper.

(20) *Cuprous iodide.*

This material was obtained from Drs Fajans and Martin as a fine suspension in water (see Fajans & Martin, 1937, for method of preparation).

(21) *Cuprous oxide.*

The fungicidal value of cuprous oxide is fully discussed elsewhere (Horsfall *et al.* 1937). The material used for tests was a suspension of cuprous oxide in cotton-seed oil emulsified with sulphite lye. The concentrate contained 20·7 % copper and 41 % cotton-seed oil. It was diluted for use to a strength equivalent to 0·1 % copper.

(iv) *Spray supplements*

The following materials were employed:

Sulphonated lorol	Gelatine
Agral 2	Lime casein
Methyl cellulose	Petroleum oil emulsion
Sulphite lye	Cotton-seed oil

Descriptions and specifications of these materials have already been given by Martin and his co-workers (Evans & Martin, 1935; Fajans & Martin, 1937).

Results

The results obtained fall into two groups:

(1) Sorting-out tests of individual materials.

(2) Tests relating to the effects of the incorporation of spray adjuvants.

Concerning the second group, certain data are available relating the results of the tests to chemical findings on the one hand and to conclusions from field trials on the other.

(i) *Sorting-out tests*

The results of tests using the rubber accelerators and other sulphur-containing compounds are given in Table I, the test spores employed being the conidia of *Venturia pirina* except where otherwise stated. In this table, a concentration of a material which, after being sprayed on a slide and allowed to dry, completely inhibited germination in the drops of spore suspension sown upon it, is termed fungicidal, represented by the symbol F. If the mean percentage germination in the test drops lay between 0 and 20, the concentration tested is considered just below

fungicidal strength (represented by f). Germinations between 20 and 60% indicate a weakly fungicidal concentration (wf) and above 60% no fungicidal value (N).

Table I

Tests with rubber accelerators and other sulphur-containing materials

All tests made with slides sprayed for 5 sec.

Material	Fungicidal value at 0·1%	0·05%	0·01%
Diphenylguanidine	—	F	f
Diorthotolylguanidine	—	—	f
Tetramethylthiuram disulphide	—	F	wf
Tetramethylthiuram monosulphide	—	—	wf
Thiocarbanilide	—	f	wf
Thiodiphenylamine	F	—	wf
Mercaptobenzthiazole	F	wf	N
Zinc diethyldithiocarbamate	F	—	N
Xanthic disulphide	—	—	N
6-ethoxy-2-aminobenzthiazole	—	—	N
Triphenylguanidine	—	—	N
Dibenzthiazyl disulphide	—	N	—
Cetyl thiocyanate	—	N	—
Lauryl thiocyanate*	—	N	—

* Tested against conidia of *Venturia inaequalis*.

The first six materials given in Table I retain a measure of toxicity even when applied at 0·01%. Of these materials the two guanidine derivatives, diphenylguanidine and diorthotolylguanidine, are slightly soluble, and thiocarbanilide is also very slightly soluble. This factor would enhance the availability of these compounds but at the same time detract from their usefulness as protectants. The remaining materials showing promise are the thiuram sulphides and thiodiphenylamine. As previously recorded, tetramethylthiuram monosulphide has been tested in the field at East Malling and showed little promise as a fungicide.

Thiodiphenylamine and diphenylguanidine were selected for submission to laboratory leaf tests using *Venturia inaequalis* as the test organism. Leaves of Crimson Cox apple were sprayed with a 0·1% suspension of each compound in 0·2% sulphite lye. The spore germinations recorded in ten drops, on six leaves sprayed with thiodiphenylamine were as follows: 72, 84, 82, 70, 82, 69, 80, 72, 78 and 74%. The figures for four drops on the four leaves, sprayed with diphenylguanidine were: 82, 70, 84, and 84%. It was concluded that neither material, at 0·1% concentration, was of sufficient fungicidal value on leaves to be given a field trial.

The other material of special interest is lauryl thiocyanate which was shown by the slide tests to be of no fungicidal value.

Table II gives the results of tests with certain copper derivatives. In a previous paper (Marsh *et al.* 1937) the distribution of fungicidal properties among a wide range of copper compounds is noted, the principal criterion used being the control of Potato Blight in field trials. Extensive field and laboratory trials have also been made with the copper oxides (Horsfall *et al.* 1937) and certain results of toxicity trials against apple scab spores with the cyanide and ferrocyanide have been recently published (Marsh, 1936). The last-named figures were extracted from a series which is given complete in Table II.

Table II

Tests with copper compounds on slides

Material	Spray exposure sec.	Fungus spores	Fungicidal value at 0·2% Cu	Fungicidal value at 0·1% Cu
Cuprous cyanide	3	*V. pirina*	*F*	*f*
,,	10	,,	—	*f*
,,	10	*V. inaequalis*	*F*	—
Cupric oxalate	10	,,	*F*	—
Cupric ferrocyanide	10	,,	*F*	—
Cuprous sulphite	10	,,	*F*	—
Cupric ammonium silicate	10	,,	*f*	—
Cuprous thiocyanate	10	,,	*N*	—
Copper phthalocyanine	10	,,	*N*	—

The first four compounds in Table II were further tested on leaves in comparison with Bordeaux mixture with the following results:

Table III

Tests with copper fungicides on leaves

All leaves sprayed for 10 sec.; conidia of *V. inaequalis* used as test spores.

Material	Fungicidal value at 0·2% Cu
Bordeaux mixture	*F*
Cupric oxalate	*f*
Cuprous sulphite	*wf*
Cuprous cyanide	*wf*
Cupric ferrocyanide	*N*

Because of its combined insecticidal and fungicidal value, cuprous cyanide was selected for a test on the field scale. The results are given in Table XI.

(ii) *Tests using spray supplements*

This section includes the results of laboratory biological tests made to examine the effects of supplements on the tenacity of spray deposits. Such effects have been determined chemically by Fajans & Martin (1937)

using cuprous iodide and cuprous oxide sprays, and comparisons with the chemical findings are given in § (iii). The majority of the laboratory biological tests have been with materials also employed in field trials and, where comparisons with the latter are possible, they are given in § (iv).

(1) *Water-soluble spray supplements.*

(*a*) *Agral* 2. Tables IV A and IV B summarize the results obtained with the use of fungicides together with Agral 2. Where the number of replications is small all the percentage germinations are given; otherwise the figures are expressed as the mean percentage and the standard error of the mean.

Table IV A

Results on slides using fungicides with Agral 2

Fungicide	Concentration of Agral 2	Fungus spores	% germination on slides: Unleached	% germination on slides: Leached
Bordeaux mixture (0·025% Cu)	None	*N. galligena*	0, 0 0, 0 0, 0	0, 0 0, 17 0, 0
,,	0·05%	,,	0, 1 0, 0 0, 0	10, 0 0, 0 6, 16
Lime sulphur 1%	None	*V. inaequalis*	0, 0 0, 0 0	0, 0 0, 0 0
,,	0·05%	,,	0, 0	0, 0

Table IV B

Results on leaves of Crimson Cox apple using Agral 2 as spray supplement

Fungicide	Concentration of Agral 2	Fungus spores	No. of results	Mean % germination on leaves: Unleached	Mean % germination on leaves: Leached
Lime sulphur 1%	None	*V. inaequalis*	35	$21 \pm 4{\cdot}8$	—
,,	None	,,	41	—	$13 \pm 2{\cdot}8$
,,	0·05%	,,	22	$20 \pm 2{\cdot}7$	—
,,	0·05%	,,	34	—	$22 \pm 4{\cdot}5$

The difference between the pair of means in the final column is $9 \pm 5{\cdot}3$, i.e. the difference is not significant. The conclusion is that the tenacity of the lime sulphur deposit on the leaves is not reduced by the presence of the Agral 2 to any extent that is recorded by the spore germination figures.

(*b*) *Sulphite lye.* Table V gives the results obtained from tests made on slides sprayed with various fungicides, with and without sulphite lye.

Table V

Results on slides using sulphite lye as supplement

Fungicide	Concentration of sulphite lye (as syrup)*	Fungus spores	% germination on slides: Unleached	Leached
Lime sulphur 1%	None	*V. inaequalis*	0	0
,,	0·75%	,,	0	16
,,	None	*V. pirina*	0	0
,,	0·75%	,,	0	68
Cuprous iodide 0·01%	None	*V. inaequalis*	6, 0	3, 0
,,	0·5%	,,	0	79
Cuprous oxide 0·1% Cu	0·15%	*V. pirina*	0, 5, 2	85, 86, 86
Bordeaux mixture 0·025% Cu	None	*N. galligena*	0, 0	17, 17
			0, 0	0, 0
,,	0·5%	,,	6, 3	71, 49
			0, 0	41, 33

* Sp.gr. 1·30, containing approx. 50% solid sulphite lye.

The deleterious effect on tenacity of 0·5–0·75% sulphite lye is reflected by the high percentage germination recorded for the leached slides on which this supplement was used. No detailed laboratory trial on leaves has been made using sulphite lye alone as a supplement, but results are given in Table VII B of leaf tests made with an oil emulsion supplement in which sulphite lye was the emulsifier.

(*c*) *Other water-soluble supplements.* Sulphonated lorol, methyl cellulose, lime casein and gelatine were tested on slides as supplements for the cuprous iodide suspension prepared by Fajans & Martin by the method described elsewhere (Fajans & Martin, 1937). The results of a comparative test are given in Table VI.

Table VI

Results on slides using various water-soluble supplements

Fungicide	Supplement	Fungus spores	% germination on slides: Unleached	Leached
Cuprous iodide 0·01%	None	*V. inaequalis*	6, 0	3, 0
,,	Gelatine 0·05%	,,	0	0
,,	Lime casein 0·05%	,,	0	0
,,	Methyl cellulose 0·05%	,,	0	20
,,	Sulphonated lorol 0·05%	,,	0	0

Under the conditions of this experiment, the use of methyl cellulose was attended by a slight loss in tenacity: the other supplements showed no adverse effect.

(2) *Oils and oil emulsions used as supplements.*

(*a*) *White oil-sulphite lye emulsion.* The emulsion employed in these tests contained 66% by volume of highly refined (Grade G) petroleum

oil, 7 % sulphite lye syrup and 27 % water. The method of compounding the emulsion is described by Kearns & Martin (1936). The results of the tests made on slides and on leaves, respectively, are given in Tables VII A and VII B.

Table VII A

White oil emulsion as supplement: results on slides

Fungicide	Concentration of oil emulsion	Fungus spores	% germination on slides Unleached	Leached
Bordeaux mixture 0·025 % Cu	None	*N. galligena*	0, 0, 0	0, 0, 0
,,	7½ %	,,	2, 3, 2	12, 73, 64

Table VII B

White oil emulsion as supplement: results on leaves

Fungicide	Concentration of oil emulsion	Fungus spores	No. of results	% germination on slides Unleached	Leached
Lime sulphur 1 %	None	*V. inaequalis*	35	21 ± 4·8	—
,,	None	,,	41	—	13 ± 2·8
,,	1 %	,,	26	14 ± 2·8	—
,,	1 %	,,	43	—	40 ± 4·7

There is a significant difference between the means in the final column—27 ± 5·5—indicating that, under the conditions of these tests, the addition of the emulsion somewhat reduced the tenacity of the lime sulphur. It should be noted that the diluted spray contained sulphite lye at a concentration of approximately 0·07 %.

(*b*) *Cotton-seed oil.* Two methods of using cotton-seed oil as a supplement have been employed in these tests. With Bordeaux mixture (Tables VIII A, VIII B) the oil as such has been added to the diluted fungicide and the mixture strongly agitated, when the Bordeaux precipitate itself acts as the emulsifier. In the tests with cuprous oxide

Table VIII A

Cotton-seed oil as supplement: results on slides

Fungicide	Concentration of oil	Fungus spores	% germination on slides Unleached	Leached
Bordeaux mixture 0·025 % Cu	5 %	*N. galligena*	0, 6, 30	0, 7, 14
Bordeaux mixture 0·1 % Cu	None	*V. pirina*	8, 4, 2	2, 0, 1
			0, 0, 0	0, 0, 2
,,	0·25 %	,,	0, 0, 0	0, 7, 6
			7, 10, 11	12, 36, 26
			0, 0, 0	1, 0, 0
			0, 0, 0	0, 0, 0

Table VIII B

Cotton-seed oil as supplement: results on leaves of Williams' pear

Fungicide	Concentration of oil	Fungus spores	No. of results	Mean % germination on leaves: Unleached	Leached
Bordeaux mixture 0·1 % Cu	0·25 %	*V. pirina*	32	2 ± 0·86	—
..	0·25 %	,,	32	—	2 ± 0·73

(Table IX), the oil was first emulsified with sulphite lye and then compounded with the oxide to give a paste containing 23 % cuprous oxide and 41 % cotton-seed oil. This was then diluted for use as required.

The results given in Table VIII A show that the addition of cotton-seed oil in these experiments had no adverse effect on tenacity. Table IX gives the figures obtained on leaves, using the cuprous oxide—cotton-seed oil emulsion at a copper concentration of 0·1 % and an oil concentration of 0·25 %.

Table IX

Cotton-seed oil emulsion as supplement: results on leaves of Williams' pear

Leaf	Spray	Exposure sec.	Fungus spores	% germination on leaves: Unleached	Leached
A	Cuprous oxide	5	*V. pirina*	15, 28	—
B	in cotton-seed	5	,,	18, 16	—
C	oil emulsion	5	,,	—	40, 54
D		5	,,	—	61, 24
E		2½	,,	66,	—
F		2½	,,	64, 68	—
G		2½	,,	—	86, 68
H		2½	,,	—	92, 87

Table IX demonstrates some loss in tenacity upon leaching the leaves sprayed with cuprous oxide-cotton-seed oil emulsion. A comparison with Table VIII B indicates the superior fungicidal value of the cotton-seed oil-Bordeaux mixture on both leached and unleached leaves.

(iii) *Comparison of results with data from chemical estimates*

From the results given in Tables IV–IX it would appear that the following materials had no adverse effect on tenacity in the experiments described—Agral 2, gelatine, lime casein, sulphonated lorol and cotton-seed oil. Sulphite lye had a markedly deleterious effect on tenacity and this effect was exhibited, to a smaller extent, by emulsions of white oil and of cotton-seed oil having sulphite lye as the emulsifier. Finally, in a single experiment, a slightly adverse effect on tenacity was shown by methyl cellulose.

For comparison of these findings with the data on tenacity determined by chemical estimation, an extract is given in Table X from the results obtained by Fajans & Martin (1937). The figures concerning the effects of supplements on the tenacity of cuprous iodide are given for the concentrations equal to those used in the biological tests.

Table X

Tenacity of cuprous iodide on a cellulose nitrate surface

Figures extracted from Fajans & Martin's Table III, 1937.

Spray supplement	Concentration %	Tenacity %
Gelatine	0·05	88
Petroleum oil emulsion	1	78
Agral 2	0·05	61
Nil	—	28
Methyl cellulose	0·05	23
Sulphonated lorol	0·05	14
Sulphite lye	0·5	2

The biological data are seen to agree generally with the findings of Fajans & Martin concerning the effect on tenacity of gelatine, Agral 2, methyl cellulose and sulphite lye. The adverse effect of sulphonated lorol, however, has not been demonstrated in a biological test, and the effects of petroleum oil emulsion, as given in Table VII, have been predominantly unfavourable to tenacity. This point is referred to further below. It should be noted, however, that in the present investigation no tests were made in which the petroleum oil emulsion was used in conjunction with cuprous iodide and cuprous oxide as in the experiments of Fajans & Martin.

The results given in Tables VIII A and VIII B showing that cotton-seed oil alone has no adverse effect on tenacity are in agreement with a number of chemical findings that this oil acts as a sticker (see Martin, 1933).

(iv) *Comparison of results with data from field trials*

(1) *Cuprous cyanide.*

The results recorded in Table III show that cuprous cyanide at 0·2% copper retained some fungicidal value on leaves but was inferior to Bordeaux mixture of the same copper concentration. An account of a field trial using cuprous cyanide against Pear Scab is given by Marsh *et al.* (1937). A summary of the results obtained is given in Table XI.

These results suggest that cuprous cyanide had proved slightly inferior to the Bordeaux mixture. It might be anticipated from the

results of the laboratory leaf test that this inferiority would be even greater than was shown in the field trial.

Table XI

Results of field trial against Pear Scab

Spray	Total fruits	Clean %	Slightly scabbed %	Badly scabbed %
Bordeaux mixture 0·1% Cu	1653	24	75·5	0·5
Cuprous cyanide 0·12% Cu	1219	19	80	1
No spray	404	0	79	21

(2) *Effects of spray supplements on tenacity.*

In the field trials listed below, the spray supplements have in general been used with lime sulphur, and their effects on tenacity have been gauged by the control obtained of Apple Scab. The full details of the experiments are to be found in the Long Ashton *Annual Reports* of the appropriate years unless otherwise stated.

(*a*) *Sulphonated lorol.* The effect of a 0·05% sulphonated lorol supplement to a lime sulphur spray programme is reflected in the figures given in Table XII on the control of apple scab.

Table XII

Percentage scab-free fruit obtained in field trial at Long Ashton, 1936

	Spray treatment		
Variety	Unsprayed	Lime sulphur	Lime sulphur + sulphonated lorol
Newton	86	99	94
Lane	83	99	99
Bramley	81	98	99
Allington	79	97	98
Cox	75	99	98
Worcester	68	81	85

It will be noted that the addition of the sulphonated lorol has not brought about any reduction in the fungicidal effect of the lime sulphur, thus corroborating the result given in Table VI.

(*b*) *Agral* 2. No figures are available concerning the effect of Agral 2 on tenacity in field trials but, in a number of experiments on apple sawfly control when this supplement has been used with lime sulphur and an insecticide, no reduction in the standard of scab control has been observed.

(*c*) *Petroleum oil-sulphite lye emulsion.* The records concerning the effect of petroleum oil-sulphite lye emulsions are assembled in Table XII. The results dated 1937, not previously published, relate to a post blossom

Table XIII

Results of field trials against apple scab using petroleum oil-sulphite lye emulsions as spray supplement

Locality	Date	Variety	Lime sulphur concentration %	Oil emulsion concentration %	Results: With lime sulphur alone		Results: With lime sulphur + emulsion	
					Total fruits	% scab-free	Total fruits	% scab-free
Hereford	1935	Derby	3	7½	5,106	95·0	7,503	95·6
,,	1935	Rival	3	7½	759	96·8	692	96·4
Long Ashton	1936	Allington	3, 3, 1*	7½, 4½, 1*	1,384	97·0	956	98·0
,,	1936	Bramley	3, 3, 1*	7½, 4½, 1*	956	98·0	2,128	79·5
,,	1936	Cox	3, 3, 1*	7½, 4½, 1*	1,256	98·6	728	97·4
,,	1936	Lane	3, 3, 1*	7½, 4½, 1*	888	99·0	644	97·2
,,	1936	Newton	3, 3, 1*	7½, 4½, 1*	296	98·3	472	97·3
,,	1936	Worcester	3, 3, 1*	7½, 4½, 1*	912	80·7	2,228	77·1
Isle of Ely	1936	Bramley	3, 1, 1	4½, 1½, 1½	689	53·8	1,216	57·3
Long Ashton	1937	Worcester	1†	1	29,689	55·8	23,764	54·1

* In successive sprayings.

† 1% lime sulphur + 0·05% sulphonated lorol.

spray on two blocks of Worcester Pearmain apples at Long Ashton which had been treated alike in the earlier sprayings.

The trials listed in Table XIII have covered a range of varieties, concentrations, degrees of scab attack and localities. In one instance, on the trees of Bramley at Long Ashton in 1936, there is an indication that the presence of the oil emulsion has lowered the level of scab control. Otherwise, the results obtained agree in showing that the oil emulsion neither increases nor decreases the fungicidal value of the lime sulphur deposit. This contradicts the conclusion drawn from Table VII that the effect of the petroleum oil-sulphite lye emulsion would be to lower tenacity.

(*d*) *Cotton-seed oil emulsion.* Table XIV gives the results of a field trial carried out on Williams' pears at Long Ashton in 1937 using the same materials and concentrations as employed for the laboratory leaf tests recorded in Table IX. These sprays were (1) a paste preparation of cuprous oxide compounded with a cotton-seed oil emulsified with sulphite lye, used at a concentration of 0·1% copper and 0·025% cotton-seed oil: (2) a cotton-seed oil-Bordeaux mixture used at the same copper and cotton-seed oil concentrations as (1). The sprays were applied to comparable blocks of trees twice before blossoming and once after. Their fungicidal value was assessed by counts of scabbed and of clean fruits made in late August.

Table XIV

Results of field trial on pear scab control using cotton-seed oil

Treatment	Total fruits	Scab free %	Lightly scabbed %	Badly scabbed %
Cuprous oxide-oil	849	19	53	28
Bordeaux-oil	570	33	43	24
No spray	421	1	23	76

It is seen that both treatments gave fair scab control, but the advantage of the Bordeaux spray over the cuprous oxide was not significant. In comparison with the Bordeaux-oil, the cuprous oxide-oil spray appeared to greater advantage in the field than in the laboratory leaf tests.

Discussion

The value of a material for use as a protectant fungicide is judged from the fungicidal effect of the dispersed residue remaining after dilution and drying. If this residue is deposited on a non-living surface such as a glass slide its retention and tenacity will be governed only by physical

factors and, with suitable precautions, a standardized method of presenting the fungicide is possible. The biological criterion of fungicidal value in general use is the effect of this deposited residue in inhibiting the germination of fungus spores. The resistance to fungicidal effect shown by spores placed in suspension on the deposit is subject to modification by such factors as temperature, age of the spores, concentration in the drop of suspension and presence of nutrient materials in the drop. The method described by Montgomery & Moore (1938) of using washed spores from cultures of standard age, suspended evenly in distilled water, gives biological testing material of a high degree of uniformity.

In the present investigation, certain departures from these levels of standardization have been made. The spores employed have been taken from naturally occurring infections in the field and have undoubtedly carried small quantities of nutrient material. This factor decreases sensitivity to fungicides and is of importance in considering the differences between the results given above in Table I and those published by Montgomery & Moore (1938). Tests have also been carried out on leaf surfaces, which again introduce variable factors having the general effect, when compared with tests *in vitro*, of necessitating an increase in the concentration of a fungicide required to inhibit spore germination. The motive in employing these methods of testing has been to explore the possibility of determining by laboratory methods the approximate strengths of fungicides required for use under field conditions.

In this connexion, special consideration has been given to the use of the laboratory leaf testing method for appraising the merits of materials put forward for use as protectant sprays against apple and pear scab. In other words, the test is directed towards evaluating the resultant of the complex series of factors determining one type of field performance of a fungicide. Each of these factors must be held constant at some arbitrary level in a laboratory test, and the value of the method can be judged only by the measure of concordance of the results with those of a comparable series of field trials. When the relation of results from a laboratory test to those from field trials is established, the test can be assigned its place in the assaying of new spray materials.

Considering in turn the factors determining protectant value of a fungicide, the factor of evenness of distribution is easily kept constant in the laboratory while, in the field, it will be adversely affected not only by variations in spray application but, also, by the persistent growth of new tissue subsequent to spraying. The amount of initial retention of a fungicide is commonly determined by the spray supplement employed

and the nature of the surface sprayed. In laboratory tests spray retention can be held constant by limiting the period of application so that the point of run-off is not reached. Where a number of wetters is being compared, and it is desired to keep the retention of fungicide constant, the amount applied must be determined by the most surface-active wetter and will be much below the run-off figure for many of the other supplements. In determining tenacity, the time, period and method of leaching used in the laboratory are all arbitrary. If resistance to leaching rises after 24 hr., the fungicide in the field would commonly have one advantage over that in the laboratory, since the likelihood is that the period between spray application and rainfall would be more than a day.

In testing availability of the spray residue in the laboratory, the approximation to field conditions is made by using the same fungus spores and leaf surfaces. In evaluating toxicity, the concentration of spores employed in the laboratory is commonly greater per unit area than would be encountered in the field, and the spores are given optimum conditions for germination. Further, it cannot be assumed that, in the field, infection in all circumstances follows germination. In these respects the conditions in the laboratory tests, as compared with those in the field, are biased against the fungicide.

Taking first the results obtained on fungicides without supplements, there is a fair measure of concordance between the results of laboratory leaf tests and field performance. Lime sulphur concentrations of 1%, and concentrations of Bordeaux mixture, cuprous oxide and cuprous cyanide equivalent to 0·1–0·2% copper, which have shown a generally high level of fungicidal value in the laboratory leaf tests, are in fact the concentrations actually effective for scab control in the field. The tendency is for the laboratory leaf tests to be somewhat less favourable to the fungicide than the field results.

This tendency is shown more markedly in the tests dealing with the effects of supplements on tenacity of fungicidal residues. An example of special interest is the effect of the petroleum oil-sulphite lye emulsion, since this product is frequently employed with insecticides in apple spraying as a supplement to lime sulphur. In the laboratory leaf tests, the effect of the emulsion was to reduce the fungicidal value of the lime sulphur deposit but in field tests it generally showed no such adverse effect.

It is suggested that this apparent discordance of results is related to the fact that the maximum possible initial retention of a spray including petroleum oil emulsion is higher than that of sprays containing wetting agents such as Agral 2 or sulphonated lorol (see Fajans & Martin, 1937).

In the laboratory tests, spray retention was kept low throughout so that the effect of a number of supplements could be compared using a constant deposition of fungicide. In the field, on the other hand, spray application is not stopped until the region of run-off is reached so that the initial deposition will, here, approach the maximum retention figure. The leaching process in the laboratory is thus applied to a much smaller quantity of fungicide per unit area than in the field. In both instances the presence of the emulsion will lead to some loss of tenacity but, in the field, it appears that the amount of spray residue initially present is sufficiently in excess of that required for protectant value to sustain this loss without perceptible reduction in fungicidal effect.

From the results obtained in the present investigation it would appear that the laboratory leaf test, carried out as described, provides a useful method of rapidly appraising the value of a fungicide for use against apple or pear scab. Any material which passes this test successfully is likely to merit field trial at a concentration equal to or lower than that employed in the laboratory. The experience in testing combined sprays suggests that with these mixtures it would be preferable to carry out a series of tenacity tests on deposits ranging in amount up to the level of maximum retention on the leaf surface.

Summary

1. Laboratory sorting-out tests on glass slides have shown the relative fungicidal value of a number of rubber accelerators and other organic sulphur derivatives. The most toxic of these materials, when tested on leaves, gave no promise of being of use in the field.

2. In laboratory leaf tests using spray supplements the adverse influence of sulphite lye on tenacity was illustrated by spore germination experiments. Loss of tenacity was similarly demonstrated in sprays including oils emulsified with sulphite lye.

3. A limited number of comparisons with field trials indicates that the laboratory leaf test may be used as an indicator of the fungicidal value of a spray material against *Venturia inaequalis* and *V. pirina*, but that in general it gives results less favourable to the fungicide than those from corresponding field trials.

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THE TOXICITY OF ETHYLENE OXIDE TO *CALANDRA ORYZAE*, *C. GRANARIA*, *TRIBOLIUM CASTANEUM*, AND *CIMEX LECTULARIUS*

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(With 11 Text-figures)

CONTENTS

Introduction

The growing importance of fumigation as a method of controlling insect pests calls for further accurate knowledge both of a particular and general nature. The former is concerned with the resistance of specific pests to various gases, while the latter deals with toxicological relationships.

Fumigation research is a comparatively new science demanding special theory and technique; but these should be developed in accordance with general physiological methods.

The object of this paper is twofold: it presents the results of investigations into the toxicity of ethylene oxide to common insect pests; also the relationships between concentration, time of exposure and mortality are investigated and compared with analogous work.

This research is essentially experimental and quantitative. The experimental method implies variation of one condition while others remain constant. The quantitative qualification means measurement and statistical treatment of the results. Before describing the experimental procedure it is proposed to survey the precautions necessary for efficient fumigation research. A considerable volume of published data is rendered unreliable through inaccurate measurement of variables and failure to maintain constant conditions. The points to be considered are:

(1) *Constant factors*:
- (*a*) Species of insect.
- (*b*) Fumigant.

(2) *Variable factors*:
- (*a*) Those influencing the amount of poison absorbed:
  - (i) Concentration.
  - (ii) Exposure time.
  - (iii) Temperature.
- (*b*) Those influencing physiological poisoning processes:
  - (i) Temperature.
  - (ii) Humidity.
  - (iii) Carbon dioxide and oxygen concentration.
  - (iv) "Pre-fumigation" effect.
  - (v) Age and stage of the insect.
  - (vi) Starvation.

(1) *Constant factors*

Species of insect and type of fumigant.

The resistance of different species and genera of insects to a given fumigant may be extremely different. Cotton (1932) has pointed out that even two species of the same genus (*Calandra granaria* and *C. oryzae*) are quite different in susceptibility to ethylene oxide.

Experience in working with one series of insects and several fumigants has revealed another interesting fact, namely, that the order of resistance may change from one poison to another (Table I).

The remarkable changes of relative resistance of the insects mentioned in Table I cannot, at present, be explained. A clue, however, is offered in the widely different effects on the behaviour of the insects caused by the three fumigants. Hydrogen cyanide causes stupor in about a minute even in low, non-lethal doses. Sulphur dioxide in severe and fatal doses causes stupor after an hour or so, while ethylene oxide

has usually no apparent effect until death occurs several days after fumigation. Possibly, with further knowledge of this kind, one may be able to classify gases by the order of resistance of species and by their effects, since presumably both depend on their mode of action. At present the main importance of the matter lies in the practical fact that caution must be observed in arguing from the results of one fumigant to another or from one insect to another.

Table I

Relative resistance of four insects to three fumigants

Fumigant	Species	Temp. °C.	5-hr. lethal concentrations (mg./l.) N.T.P.	Author
Hydrogen cyanide	*Calandra granaria*	25	14·00	Peters & Ganter (1935*a*)
	C. oryzae		12·00	Allison (1928)
	Tribolium castaneum		0·36	Bovingdon (1935)
	Cimex lectularius		0·17	Bovingdon & Busvine (1936)
Ethylene oxide	*Tribolium castaneum*	25	27·00	This paper (99 % lethal dose)
	Cimex lectularius		12·30	
	Calandra granaria		8·40	
	C. oryzae		4·10	
Sulphur dioxide	*Calandra oryzae*	20	10·80	Busvine (1936)
	Tribolium castaneum		9·70	
	Calandra granaria		8·30	
	Cimex lectularius		5·90	

(2) *Variable factors*

(*a*) *Influencing the amount of poison absorbed.*

Insects are dosed with fumigant poisons by the simple method of subjecting them to a constant concentration for a definite time. The maintenance of constant physical conditions has been achieved in this work by the use of the fumigation apparatus designed and described by Bovingdon (1934) and the difficulties in accurate measurement of small quantities of ethylene oxide were largely overcome by the technique developed by Lubatti (1932, 1935).

(i) *Concentration.* The maintenance of a constant concentration of gas over experimental insects presents several difficulties. In large fumigation chambers it is often found that the concentration falls considerably during the experiment because of leakage and absorption. In smaller glass vessels the difficulty of accurate dosage is increased, and there is still the possibility of bad distribution of gas unless adequate stirring devices are installed. Experiments with sulphur dioxide and ethylene oxide may be quoted to illustrate this (Tables II and III).

Table II

Gas: Sulphur dioxide. Vessels: 7–8 l. desiccator. Temperature: 20° C. Method of stirring: Swinging suspended card. Method of sampling: Capillary to evacuated flask containing iodine solution. Titration against thiosulphate.

Sample after (min.)	% expected concentration found			
	Top	Middle	Bottom	
1–2	29·5	97·5	236	Not stirred
8	75·5	97·0	143	,, ,,
65	88·5	96·0	96	,, ,,
2–3	95·5	97·5	104	Stirred
2–3		100·5		,,
2–3		97·0		,,
2–3		98·5		,,
		Av. = 98·5		

In order to eliminate the possibilities of error from bad distribution of gas, the ethylene oxide-air mixture was stirred about fifty times before the commencement of each experiment. Inspection of Table III shows that this should be ample.

Table III

Gas: Ethylene oxide. Vessel: Flask of Bovingdon's apparatus (4 l.). Temperature: 25° C. Method of stirring: Agitation of a plunger. Method of sampling: Evacuated flask to sampling connexion. Estimation by Lubatti's technique.

Sample after min.	Number of stirs	% expected concentration found
10	0	430
30	0	214
40	0	194
230	0	156
10	0	430
10	5	178
10	12	114
10	25	100
10	50	100
10	75	102

In order to keep a check on possible leakage and to increase accuracy of dose estimation, a sample was taken at the beginning and end of each experiment.

The comparatively small size of the fumigation flask in Bovingdon's apparatus (about 4 l.) made it possible to draw only small samples for analysis. Accordingly flasks of about 130 c.c. were used, each containing a standard quantity of magnesium bromide-sulphuric acid absorbent and provided with a capillary tube with a tap. The flasks were evacuated to a known pressure, and connected to the sampling connexion of the apparatus by the capillary tube. The tap was then opened and the flask

left in connexion with the apparatus for 5 min. to allow the gas drawn to reach room temperature. Titration was done about an hour after drawing the sample. A number of check experiments showed that if a very short time was allowed, the absorption was not complete. On the other hand, with a long delay there was a tendency for the absorbent to weaken by loss of hydrogen bromide. The titration was done with 0·05 N solution of sodium hydroxide in a micro-burette and comparison with a blank indicated the amount of absorbent which had reacted with ethylene oxide. From this, the concentration of the gas was calculated and reduced to N.T.P.

The concentrations measured ranged from about 1 to 100 mg./l. The estimated accuracy in determination amounted to 0·30 mg./l. This corresponds to an error of 30% at 1 mg./l., and 0·30% at 100 mg./l. The large error was to some extent reduced by taking the average of three determinations when the doses were low. These low concentrations occurred only in the 20 hr. exposures.

As a general rule very little variation in adjacent samples was observed. There was no appreciable tendency to leakage, and the circulating and stirring devices in the apparatus maintained good gas distribution in the main flask. The average difference in the usual two samples was about 3%.

(ii) *Time of exposure.* The time of exposure in all experiments was measured simply by wrist watch. The inaccuracy involved in measurement was practically negligible since an error of ½ min. amounts to only 1·66% with the shortest exposure. The difficulty of measuring exposure time is due to the fact that the insects cannot be exposed to the full concentration instantaneously.

The 60 c.c. animal chamber of Bovingdon's apparatus was flushed through with twice its volume of air-gas mixture from the reserve flask. It was then turned into communication with the main flask and the circulation pump started. Even with these precautions, there is a definite lag before full experimental concentration is attained. This is shown by Table IV, which gives results of special test samples taken directly from the animal chamber.

Table IV

Concentration in the animal chamber

Period before sample (min.)	0	5	10	20
% final concentration	78	87·8*	94·5*	98

* Average of two experiments.

By integrating a curve based on Table IV, the effect of the lag may be estimated as being equivalent to a complete hiatus of 1·6 min. (assuming concentration × time for a given effect to be constant).

(iii) *Temperature and its effect on physical constants.* With high boiling point vapour poisons (e.g. orthodichlorobenzene B.P. = 176° C.), a rise in temperature even within the biological range may increase considerably the vapour pressure. As a result the saturation concentration becomes greater, and higher doses become available. This does not apply to low boiling point fumigants such as ethylene oxide, hydrogen cyanide or sulphur dioxide since saturation concentrations are never approached.

Two other physical properties, influenced by temperature, are concerned with poison uptake. With a rise in temperature the rate of *diffusion* of gases is increased while *sorption* is decreased. The temperature coefficients of diffusion of gases are extremely close to unity. Those dealing with solution and adsorption of gases are almost invariably fractional. The values of Q_{10} for $\frac{1}{\text{lethal dose}}$ at different temperatures,[1] on the other hand, are usually between 1·5 and 5 (see Table VI). It seems, therefore, that in so far as the effect of temperature is concerned neither diffusion of gas in air nor absorption by the insect plays a predominating part in regulating the rate of poisoning of insects by fumigants. (These observations refer only to experimental conditions in which the insects are surrounded by a standard concentration of gas. In these circumstances diffusion only refers to passage of gas along the tracheae of the insect.)

The effects of temperature on toxicity appear to be bound up with chemical poisoning processes and also with the physiology of the insect which will be considered later.

(*b*) *Factors influencing the poisoning process.*

The action of a poison is to dislocate the normal metabolism of an organism and, therefore, it is not surprising that the degree of poisoning is closely bound up with the physiological condition of the organism. For this reason chemical methods of pest control would benefit by elucidation of certain physiological aspects of biology which have been comparatively neglected in the past.

The salient points in the relationship between the physiological condition of insects and their resistance to poisons can best be illustrated by means of a table (Table V).

[1] The provisional use of Van't Hoff's coefficient in this connexion is justified on p. 611. The introduction of a more valid temperature coefficient awaits a more thorough understanding of toxicological processes.

Table V

Physiological condition and resistance

Cause	Physiological effect	Toxicological effect
Raising temperature	Increases metabolism (e.g. respiration): Vernon (1897), Crozier (1924), Batelli & Stern (1913), Sayle (1928), Krogh (1914), Rodgers (1929), Bodine (1921)	Increases susceptibility: Jones (1933), Cotton (1932), Peters & Ganter (1935*a*), Bovingdon & Busvine (1936)
Varying relative humidity	No pronounced effect on metabolism: Rivnay (1932), Mellanby (1936)	No pronounced effect on susceptibility: Brinley & Baker (1927), Lindgren & Shepard (1932)
Addition of carbon dioxide	Increases respiratory movements: McGovran (1932). Opens spiracles of insects: Hazelhoff (1927), Wigglesworth (1935)	Increases susceptibility to non-stupefying gases: Cotton & Young (1929), Pratt, Swain & Eldred (1933)
Lowering oxygen content of the air	Increases respiratory movements: v. Buddenbrock & Rohr (1923), Babak & Foustka (1907). Opens spiracles more often: Wigglesworth (1935)	Increases susceptibility (to various gases): Cotton (1932)
Small preliminary dose of hydrogen cyanide	Lowers respiratory rate and causes stupor: Buchanan (1926), Dixon & Elliot (1929), Child (1919), Hyman (1916), Shafer (1911), Allen (1911)	Decreases susceptibility (to hydrogen cyanide): Gray & Kirkpatrick (1929), Pratt, Swain & Eldred (1931)
Age and stage of insect	Order of respiratory rate: adult→larva→pupa: Batelli & Stern (1913), Ludwig (1931)	Order of susceptibility: adult→larva→pupa: Cotton (1932)
Starvation	Respiration rate and R.Q. decrease: Fink (1925), Child (1919), Cook (1932), Bodine (1921)	Susceptibility to ethylene oxide decreases: Mayer (1934) and this paper. Susceptibility to hydrogen cyanide increases: Bovingdon & Busvine (1936)

The purpose of this work is to deal mainly with the relationship between mortality, dose and time, other factors being constant. However, it will be necessary to consider briefly the importance of the various influences of metabolism and to assess the efficiency with which they have been stabilized.

(i) *Temperature.* The fact that the resistance of insects decreases with a rise in temperature is well known, but very little accurate work has been done to determine the magnitude of the effect. The quantitative effect on normal physiological processes has been referred to three types of formula:

(1) According to Krogh (1914) the relation is linear within the normal biological range.

(2) Van't Hoff's equation $\frac{\text{Rate at } t+10}{\text{Rate at } t}=\frac{Q}{10}$ has been widely used, but the value Q_{10} is not a constant. It usually decreases from about 4 at 10° C. to about 1·5 at 30° C.

(3) Arrhenius has proposed a formula:

$$\frac{V_1}{V_0} = e^{\frac{\mu}{2} \frac{(T_1 - T_0)}{(T_1 T_0)}}$$

(V_0 = velocity at temperature T_0, V_1 = velocity at temperature T_1), which allows for this fall in Q_{10}. However, two or three values of his constant μ are usually required to fit different portions of the temperature range.

Neither the linear relation nor Arrhenius's formula expresses the relation between temperature and toxicity. The effect can be expressed provisionally by values of Q_{10} corresponding to Van't Hoff's coefficient, which is convenient because of general use.

In place of velocity $\left(=\frac{1}{\text{time}}\right)$, the toxicity can be measured as $\frac{1}{\text{concentration}}$ for a predetermined kill. The increase in toxicity corresponding to a 10° rise in temperature can be calculated for any results at two or more temperatures from the formula:

$$Q_{10} = \left(\frac{C_1}{C_2}\right)^{\left(\frac{10}{T_2 - T_1}\right)}$$

(C_1 = lethal concentration at T_1, C_2 = lethal concentration at T_2).

Table VI shows values calculated in this way from data of different workers. These results show an unfortunate lack of uniformity, which makes it impossible to generalize.

That this is not due to different experimental methods or different insects is shown by the great difference in the Q_{10} values of ethylene oxide and hydrogen cyanide towards *Calandra* obtained by the same technique. Such discrepancies are reminiscent of the conclusions of Hartmann (1918), who, working with *Cladocera* in toxic solutions, found no uniformity in temperature coefficients and concluded that their changes in value indicated different complex processes (e.g. osmotic pressure, ionization etc.) underlying the toxic action of different poisons. The various processes are probably dominant to different extents according to the position on the temperature scale.

The present series of experiments were all done at 25° C. From the limited evidence available, the value of Q_{10} would be expected to be about 2·5. The variation in the temperature of the fumigation cabinet did not exceed $\pm 0{\cdot}15°$ C. (Bovingdon, 1934). On the above assumption this corresponds to a dose error of $\pm 1{\cdot}4\%$.

(ii) *Relative humidity.* The work of Lindgren & Shepard (1932)

Table VI

Values of Q_{10} for fumigants

Authors	Insect	Fumigant	Exposure range hr.	Temperature range (°C.) 0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 — Values of Q_{10}
Wilmot & Walker (1932)	*Mineda juglandis*	Hydrogen cyanide	½	← 3·24 →
Cotton (1932)	*Tribolium confusum*	Ethylene oxide	3	← 3·33 →← 3·46 →
Jones (1933)	*Limonius* spp.	Carbon disulphide	5	← 1·2 →← 1·72 →← 1·78 →← 2·03 →← 1·31 →
Peters & Ganter (1935*a*)	*Calandra granaria*	Hydrogen cyanide	2½–30	← 0·48 →← 1·04 →← 1·59 →← 1·88 →← 2·18 →
Peters & Ganter (1935*b*)	*C. granaria*	Ethylene oxide	2½–30	← 5·18 →← 2·40 →← 1·95 →← 1·11 →
Bovingdon & Busvine (1936)	*Cimex lectularius*	Hydrogen cyanide	⅙–2½	← 2·7 →← 4·7 →
Parkin & Busvine (1937)	*Lyctus* sp.	Hydrogen cyanide	1–6	← 2·47 →
Lindgren (1935)	*Tribolium castaneum*	Carbon disulphide	5	← 3·2 →← 1·28 →← 1·45 →← 2·05 →← 1·90 →
		Chlorpicrin	5	← 2·63 →← 1·45 →← 1·80 →← 2·47 →← 2·42 →

suggests that, over a wide range, relative humidity has little effect upon resistance of insects to fumigants.

In all the experiments carried out the air was conditioned to about 45% R.H. by passing it through solutions of potassium hydroxide. The amount of variation, observed by an "Edney" paper hygrometer, was well within $\pm 5\%$ and therefore could be neglected.

(iii) *Influence of carbon dioxide and oxygen pressure.* Neither effect has been studied in this work and their influence is excluded by using normal air deprived of all carbon dioxide by the conditioning potash solutions.

(iv) "*Prefumigation*" *effect.* Since ethylene oxide does not cause stupefaction, the possibility of small doses causing a fall in the rate of metabolism is unlikely and the design of Bovingdon's apparatus is such that the insects are exposed to almost the full concentration of the gas at the commencement of the experiment.

(v) *Influence of age and stage of insect.* The close dependence between rate of metabolism and susceptibility is supported by indirect evidence provided by the different stages of insect as pointed out by Cotton (1932). The order of metabolic rate: adult →larva →pupa has been paralleled by a similar order of susceptibility.

There are, however, a few exceptions which indicate that other factors besides metabolic rate come into question. For example, *Lyctus* larvae are apparently more susceptible than the adults (Parkin & Busvine, 1937). Also work on the bed-bug has shown that the order of susceptibility to ethylene oxide, hydrogen cyanide and orthodichlorobenzene is: eggs→young nymphs→adults→4th and 5th stage nymphs, but towards sulphur dioxide, and tri- and perchlorethylene, the eggs are about twice as resistant as the adults and old nymphs.

In the present series of experiments only adults of the stored product pests were used and only 4th and 5th nymphal bed-bugs. Apart from this no special precautions were taken to ensure that the insects were exactly of the same age. The beetles were chosen at random from healthy cultures.

(vi) *Starvation.* The effect of starvation was only studied in *Cimex* where it is of practical importance. Bugs can endure long periods of starvation and take relatively enormous meals of blood, and it is not surprising that their resistance varies at different times.

Their resistance to hydrogen cyanide decreases from the first day after feeding, though immediately after the meal it is low, probably because of the large amount of water in the food which may absorb the soluble gas.

The relation between starvation and resistance of bugs to ethylene oxide is entirely different, for, as pointed out by Mayer (1934), starved bugs are more resistant than recently fed ones. This is, perhaps, due to the decreased rate of metabolism which seems to occur in starving invertebrates (Fink (1925), *Leptinotarsa* and Bodine (1921), locusts). The different effect of hydrogen cyanide recalls the fact that this gas is exceptional in not being influenced by addition of carbon dioxide (Cotton, 1932). These anomalies may be due to the sudden stupefying action of hydrogen cyanide which probably obscures minor influences upon metabolism.

The enhanced resistance to ethylene oxide does not increase in a linear fashion. Fig. 1, which shows medium lethal doses (calculated

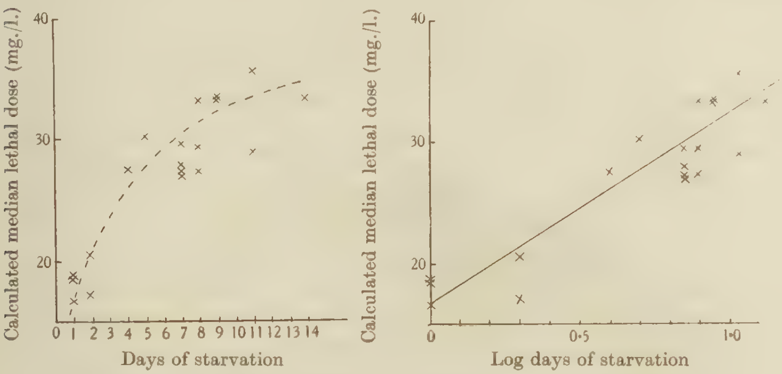

Fig. 1. Resistance of *Cimex* after different periods of starvation

from a number of experiments and corrected for control mortality) plotted against the logarithm of the starvation period, suggests that the relationship is logarithmic.

For practical purposes resistance may be said to be highest 7–12 days after starvation at 25° C. and bugs in this condition have been used almost exclusively in the present experiments. After about 14 days the deaths from starvation more than compensate for the enhanced resistance.

Experimental procedure

(1) *Planning of experiments*

(*a*) *Arrangement.*

The experiments with ethylene oxide were planned to provide concentration-mortality regression lines for each insect at a number of different exposures. Data obtained in this way were intended to show

time-concentration relationships for a constant effect (mortality). It was expected that the relation would be of the form:

$$c^n t = K,$$

(c = concentration, t = time, c and K = constants),

or $$n \log c + \log t = \log K.$$

Therefore the exposure times were chosen, so far as was practicable, at constant logarithmic intervals. The selected times were ½, 1, 2, 4, 6, 9 and 20 hr.

(*b*) *Accuracy.*

In order to gather greatest information about time-concentration relationships it is desirable to obtain data over as wide a range as possible but, unfortunately, at either extremity of the curve a decrease in accuracy sets a limit to useful exploration. This is illustrated by Table VII. With long exposures the concentrations are low and the percentage error of estimation becomes high. The accuracy was to some extent improved, however, by taking the average of two and sometimes three determinations for each experiment. The only way to obtain uniform accuracy throughout would be by carrying out many more long exposure experiments and this was not found practicable.

The limitation of the short exposures was not due to a reading error which, as explained before, is negligible. The difficulty here is due to the lag in attaining full concentration. From calculations based on Table IV (assuming $c \times t \simeq$ constant) the lag is approximately equivalent to a complete hiatus of 1·6 min. This is about 5% of a half-hour exposure, and it was decided that shorter exposures would involve too great and too uncertain an error. A correction was not introduced, since it would involve too many assumptions.

Table VII

Percentage accuracy of time and concentration

Estimated accuracy of measurement		Exposure time (hr.)					
		½	1	2	4	6	20
1/3 mg./l.	% M.L.D. of *Tribolium*	0·56	0·71	1·30	2·12	2·56	6·15
	% M.L.D. of *C. granaria*	0·92	1·92	3·06	5·80	8·4	19·2
	% M.L.D. of *C. oryzae*	1·25	2·75	5·6	10·6	13	56
1·6 min. lag	% time of exposure	5·3	2·67	1·34	0·67	0·45	0·13

M.L.D. = median lethal dose.

The experimental range is, therefore, limited by the lag in exposures shorter than ½ hr. and the low concentrations found in exposures greater

than 20 hr. Within this range the accuracy is variable, but this will find expression in the standard error of individual regression lines and can be allowed for in a statistical analysis of the results.

(2) *Treatment of insects*

The stored product insects were taken from cultures in 7 lb. jars containing appropriate food (grain for *Calandra granaria*, rice for *C. oryzae* and wholemeal flour for *Tribolium*). New cultures were used whenever signs of overcrowding, moulds, or mites were observed.

The bed-bugs were kept in muslin-topped glass tubes and fed once a week on the ears of a lop-eared rabbit.

All the insects were reared at 25° C. and about thirty insects were used in each experiment. They were exposed to the fumigant in small muslin bags and afterwards transferred to clean glass tubes. Suitable food was added and the tubes were corked and kept in an incubator at 25° C. A number of special experiments demonstrated that corking the tubes after fumigation did not have any harmful effects.

(3) *Estimation of mortality*

There are several difficulties in ascertaining mortality of insects after fumigation and these can only be overcome by periodic observations over a considerable period following the experiment. The primary difficulty is to distinguish which insects will finally succumb. In the first place the toxicological effect is graded and not absolute as every state can usually be found between slightly affected to moribund and dead insects. The affected insects sometimes remain partially paralysed for long periods before final death or recovery. A further difficulty is introduced by the stupefying effects of some gases. Immobility may be caused almost at once (hydrogen cyanide) or after about an hour (orthodichlorobenzene, etc.) and the duration of the stupor may be a matter of hours or even extend to weeks. The period of greatest rate of revival of bed-bugs after exposure to hydrogen cyanide or orthodichlorobenzene is 2–4 days after the experiment (at 25° C.). After about 7–10 days, revival of the great majority of bugs has occurred. *Lyctus* larvae on the other hand have been observed to recover after 17 days at 25° C. and 21 days at 20° C. during which they showed no signs of life (Parkin & Busvine, 1937).

The rate of revival is related to the severity of the exposure. If experiments are grouped according to the percentage of recovery it

is evident that the more severely affected insects (85–99% kill) do not recover as quickly as less heavily dosed ones (70–84% kill) (Table VIII).

Table VIII

Rate of revival of bed-bugs after fumigation with HCN at 25° C. Expressed as the percentage of maximum revival in each class

Mortality range of experiments	Days after experiment 1	2	3	4	5	6	7	8	9	10	11	12	
85–99%	4	5	—	27	—	75	—	100	—	100	—	94	% max.
70–84%	28	—	40	—	80	94	100	—	100	—	91	—	revival

Ethylene oxide introduces the problem of a delayed death in place of a partial revival. The mortalities in the present work were estimated in each experiment when the rate of deaths had decreased to the normal low rates observed in controls. (*Calandra oryzae* and *C. granaria* 2–4% and *Tribolium castaneum* about 5% in 7–10 days.)

The rate with *Cimex* constitutes an exception owing, no doubt, to the fact that bugs were not fed for at least a week before the experiment nor during the subsequent period of examination. The control deaths amounted to approximately 1% after a week's starvation, 9% after 2 weeks and 21% after 3 weeks. A correction was made by estimating the true kill by Abbott's formula:

$$\text{True kill} = \frac{100\,(x-y)}{100-y},$$

where x = observed % kill and y = % control deaths.

The correction was adjusted in each experiment according to the total period of starvation.

The duration of the interval before death is related to the severity of exposure, the greatest rate of death occurring earlier with more

Table IX

Percentages of final mortalities on different days after fumigation with ethylene oxide

Insect	Mortality range %	Days after the experiment 1	2	3	4	5	6	7	8	9	10	11
Calandra oryzae	1–49	—	9	40·0	59	67	80·5	80·5	95	97	100	100
	50–99	—	69	79·0	84	92	95·0	96·0	94	99	100	—
C. granaria	1–49	—	44	72·0	68	90	93·5	95·5	96	97	100	100
	50–99	—	77	79·5	93	91	96·0	97·0	99	100	100	—
Cimex lectularius	1–49	—	63	75·0	91	94	97·0	97·0	100	100	—	—
	50–99	—	90	95·0	97	98	99·0	99·0	100	—	—	—
Tribolium castaneum	1–49	60	89	100·0	89	100	—	—	—	—	—	—
	50–99	91	100	100·0	—	—	—	—	—	—	—	—

severely dosed individuals. (This is the converse of the relationship obtaining with revival from stupefying gases.)

The length of the delay also depends upon the species of insect. Both these facts are illustrated by the analysis of mortality presented in Table IX.

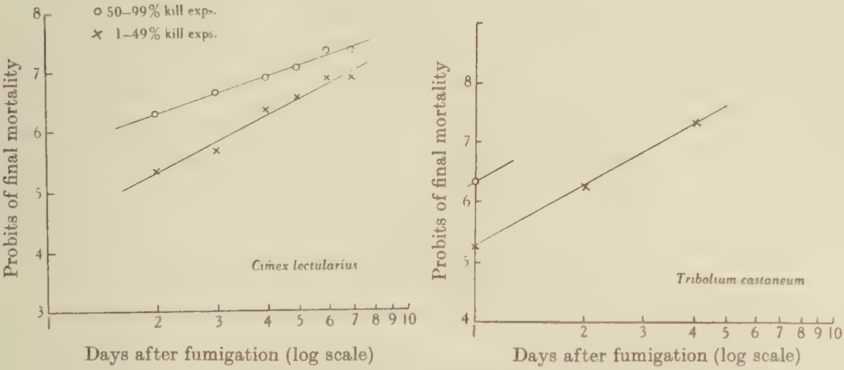

Fig. 2. Incidence of mortality on different days after fumigation.

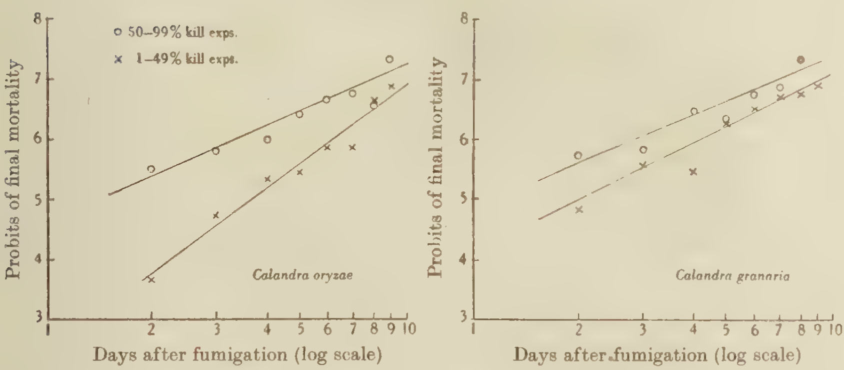

Fig. 3. Incidence of mortality on different days after fumigation.

A further interesting point emerges from the data if the mortalities given in Table IX are converted into probits to correct for random variation. If these values are plotted against the logarithm of the time after experiment it will be found (Figs. 2 and 3) that the relation is linear. This is in harmony with the observation of Bliss & Broadbent (1935), that recoveries of blow-flies stupefied with hydrogen cyanide give a symmetrical distribution, if the stupefaction time is measured in

logarithms. In both instances the time measured is that for a chain of biochemical processes. With the recovery from stupefaction it must deal with elimination of poison, while with ethylene oxide it probably depends upon conversion of the poison into an active form, possibly:

$$\text{Ethylene oxide} \underset{(\textit{hydrolysis})}{\rightarrow} \text{ethylene glycol} \underset{(\textit{oxidation})}{\rightarrow} \text{oxalic acid.}$$

Results

The results may be classified under two toxicity relationships. The data obtained at each exposure time give concentration—effect (i.e. percentage kill) regression lines and the whole series provide a time-concentration relation for a constant effect (percentage kill).

Both types of results can be regarded from various standpoints. In the first place it might be thought that the equations expressing inter-relations might contribute information about the fundamental processes of poisoning. Followers of Arrhenius have pointed out similarities between physiological equations and those expressing physico-chemical phenomena. For example the effect of temperature on toxicity is compared to its effect on the rate of chemical reaction (Reiner, 1933). Again the relation between concentration and time is compared with so-called "mono-molecular" reactions (Arrhenius, 1915). However, as Clark (1933) has pointed out, such similarities are probably superficial and misleading. The chief reason for this view lies in the extreme complexity of biological processes. If physicists hesitate to deal with the more complex forms of, for example, adsorption, it is apparent that physiology and toxicology are far beyond fundamental explanation at present. Apparently simple relations may be due either to a large number of contributing causes cancelling each other out, or else to some simple process (e.g. diffusion) being much slower than the others. In either case the relations of the whole chain of processes will be regulated by one, possibly simple, link.

The second reason for our inability to deduce much from toxicological results is the variability of biological material which makes it possible to fit several simple equations over the greater part of most curves.

It seems therefore that the chief value of relationships deduced from the data will be the empirical one of standardization of entomological fumigation results on the lines of analogous toxicological work. In spite of the extent of fumigation literature there have been very few attempts to do this. The main contributions are those of Strand (1930), Bliss (1934) and Bliss & Broadbent (1935). Until fumigation curves are expressed

in a simple general form it is impossible to find reliable criteria for resistance and toxicity which are naturally of considerable practical importance.

(1) *Concentration-mortality relationships*

It has been known for a considerable time that concentration-mortality curves are asymmetrically sigmoid in shape. As a result of this, it is not easy to use all the data in drawing the best possible curve,

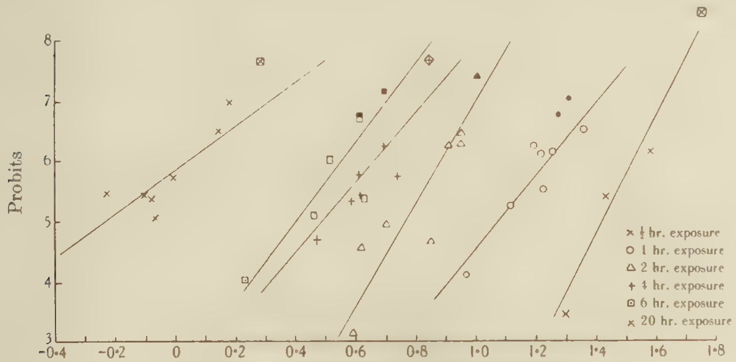

Fig. 4. Dose-kill regression lines for *Calandra oryzae*.

Table X

Calandra oryzae *results*

Exposure time hr.	Average no. of insects per experiment	Regression equation ($x = \log c$; $y =$ probits)	Degrees of freedom	χ^2	Probability $P>0{\cdot}05$* $P<0{\cdot}05$** $P<0{\cdot}01$***	50% kill concentration mg./l.
½	31·0	$y=9{\cdot}4773x-8{\cdot}4804$	1	4·1162	**	26·4400
1	25·2	$y=7{\cdot}4328x-3{\cdot}0445$	7	9·8117	*	12·0900
2	25·1	$y=6{\cdot}8622x-0{\cdot}2930$	6	23·8736	***	5·9060
4	27·1	$y=6{\cdot}2214x-1{\cdot}9184$	5	23·2401	***	3·1280
6	34·6	$y=6{\cdot}5603x+2{\cdot}3327$	5	20·6898	***	2·5510
20	27·7	$y=3{\cdot}6207x+5{\cdot}8322$	7	11·9826	*	0·5891

and the 99% kill position is, in particular, difficult to define. Accordingly, the 50% kill position (median lethal dose) was used as being the most reliable for comparative purposes (Tattersfield & Morris, 1924; Trevan, 1927; Strand, 1930).

A considerable contribution was made by Bliss (1934, 1935) who corrected the observed kills for random variation assuming a normal

distribution. He used as units "probits" equal to the normal standard deviation of mortality. To correct for the asymmetry still observed, he plotted the dose in logarithms. Whatever the fundamental causes, this gives a good fit for most similar data over the higher range of doses. At low doses discontinuity is sometimes observed. O'Kane *et al.* (1934) claimed to have eliminated this discontinuity by using *logarithms* of

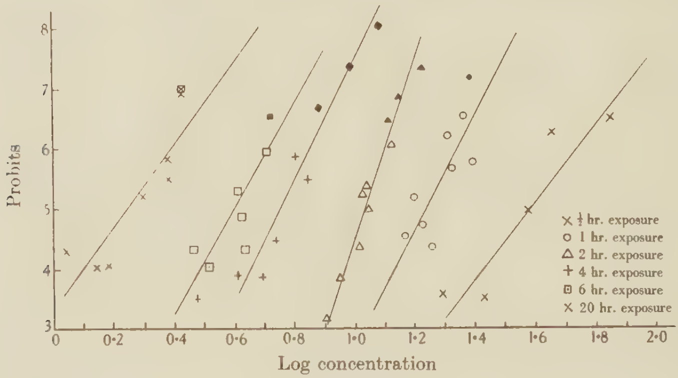

Fig. 5. Dose-kill regression lines for *Calandra granaria.*

Table XI

Calandra granaria *results*

Exposure time hr.	Average no. of insects per experiment	Regression equation ($x=\log c$; $y=$probits)	Degrees of freedom	χ^2	Probability $P>0{\cdot}05$* $P<0{\cdot}05$** $P<0{\cdot}01$***	50% kill concentration mg./l.
½	27·6	$y=6{\cdot}6091x-5{\cdot}5499$	3	3·6172	*	39·480
1	29·0	$y=9{\cdot}4694x-6{\cdot}7253$	8	30·8859	***	17·310
2	34·6	$y=14{\cdot}2982x-9{\cdot}8095$	8	13·0721	*	10·860
4	34·7	$y=10{\cdot}0265x-2{\cdot}5902$	7	21·2891	***	5·715
6	38·6	$y=8{\cdot}5795x-0{\cdot}1420$	5	27·5350	***	3·975
20	33·1	$y=6{\cdot}8607x-3{\cdot}3304$	7	29·4396	***	1·752

probits against logarithms of doses. Bliss (1935), however, comments that a logarithm of a probit is not a natural function. The same effect could have been obtained with an exponential equation. However, he claims that a logarithmic equation fits all but the lowest doses as well as exponential or hyperbolic functions and with one loss constant. Moreover, the logarithmic formula gives the highest 99% theoretical dose and hence, as a practical consideration, the greatest margin of safety.

Because of these advantages and because it was found to give a reasonably good fit with the data presented in this report, which mainly deals with the higher dose range, Bliss's method has been employed for the statistical analysis of results.

The results may be seen plotted graphically in Figs. 4–7, and the essential statistics of each set of data are set out in Tables X–XIII.

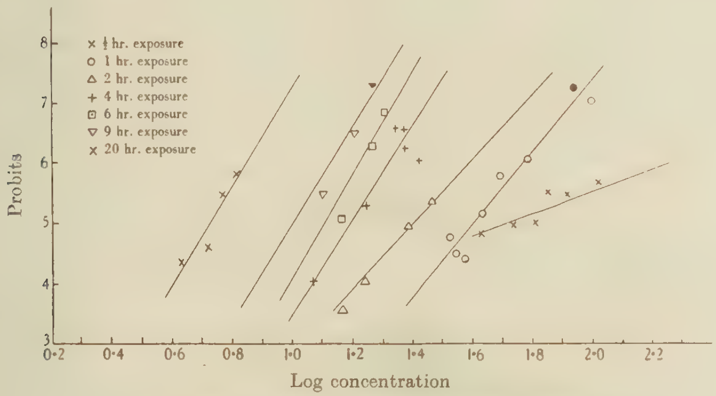

Fig. 6. Dose-kill regression lines for *Tribolium castaneum*.

Table XII

Tribolium castaneum *results*

Exposure time hr.	Average no. of insects per experiment	Regression equation ($x = \log c$; $y =$ probits)	Degrees of freedom	χ^2	Probability $P > 0\cdot05$* $P < 0\cdot05$** $P < 0\cdot01$***	50% kill concentration mg./l.
½	31·5	$y = 1\cdot8876x + 1\cdot727$	4	3·5527	*	54·190
1	35·7	$y = 6\cdot1578x - 4\cdot909$	6	7·3399	*	40·660
2	25·5	$y = 5\cdot4921x - 2\cdot716$	2	0·0906	*	25·400
4	30·1	$y = 7\cdot7653x - 4\cdot281$	4	7·9298	*	15·680
6	29·7	$y = 8\cdot6603x - 4\cdot634$	1	0·1821	*	12·950
9	31·0	$y = 10\cdot5494x - 6\cdot196$	1	0·1173	*	11·520
20	34·0	$y = 8\cdot3650x - 1\cdot124$	2	3·5414	*	5·396

I am indebted to Dr Bartlett, statistician at Jealott's Hill Agricultural Station for the analysis of the results and for his comments.

The number of experiments at each exposure is equal to the number of degrees of freedom plus two. (One experiment being used up to fix the position and one the slope of the regression line.)

In the probability column, the level of P (indicated by asterisks) gives a measure of the uniformity of the insect populations.

Values below 0·05, found with each insect except *Tribolium*, indicate unexplained heterogeneity.

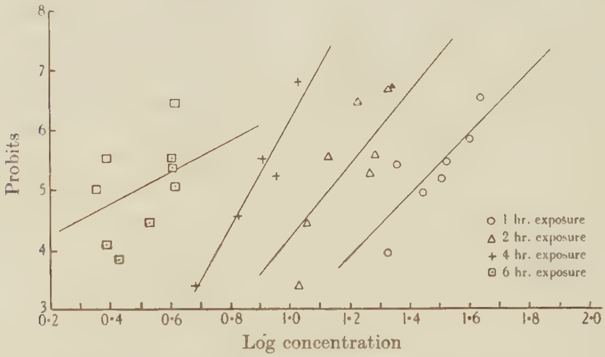

Fig. 7. Dose-kill regression lines for *Cimex lectularius*.

Table XIII

Cimex lectularius *results*

Exposure time hr.	Average no. of insects per experiment	Regression equation ($x=\log c$; $y=$probits)	Degrees of freedom	χ^2	Probability $P>0{\cdot}05$* $P<0{\cdot}05$** $P<0{\cdot}01$***	50% kill concentration mg./l.
1	21·6	$y=5{\cdot}0743x-2{\cdot}1828$	5	16·0273	***	26·03
2	24·5	$y=7{\cdot}0854x-3{\cdot}0401$	6	32·9310	***	13·63
4	28·2	$y=8{\cdot}8641x-2{\cdot}7361$	3	6·1033	*	7·40
6	24·8	$y=2{\cdot}6475x+3{\cdot}6934$	7	35·6051	***	3·12

(2) *Concentration-time relationships*

The relation between exposure time and concentration of fumigant to produce a constant mortality can be expressed by a simple hyperbolic equation as a first approximation:

$$c.t=W \text{ (Haber's formula).}$$

Two types of divergence from this equation have been claimed, one at low concentrations and one for short exposures, both resulting in increased values of W. It has already been stressed that certain common defects in technique produce similar results, and the divergences cannot be accepted as fundamental unless these errors have been eliminated.

A deviation at low concentrations beyond the limits of error has been observed by several workers. The $c.t$ curve may be said to flatten out. It is presumed that this is due to neutralization or excretion of the poison by tissues of the animal investigated. Assuming that the rate of elimina-

tion of poison is constant, the $c.t$ curve will approach asymptotically the highest tolerated concentration (c_0) instead of zero.

$$(c-c_0)\,t=W \text{ (Flury, 1921).}$$

This toleration effect, however, is by no means universal. Flury (1921) observed it with hydrogen cyanide but not with phosgene and suggested that the latter belongs to a class of gases which cannot be eliminated. Analogous results were found in the work on various poisons to fishes. Powers (1917) found theoretical threshold concentrations for most poisons to goldfish, but some metallic salts were exceptional. Carpenter (1927) showed that heavy metallic salts in general gave no indication of tolerated concentrations, and pointed out that their mode of action was peculiar. (They were not taken into the body, but acted by forming a film over the gills.)

The other deviation from the hyperbolic relation is noted in short exposures to high doses. This is treated in different ways by different authors. Hartmann (1918), working with *Cladocera* in solutions of inorganic salts, introduced a small time-toleration constant ("zeitlich Entgiftungsfaktor" $=t_0$) as well as a concentration tolerance factor. His formula, founded on a number of quite plausible assumptions, contains, however, no less than four constants. It is too cumbersome and not sufficiently well established for general application:

$$(c-c_0)\,(1-e^{-n(t-t_0)})=W,$$

$c_0=$ threshold concentration, $t_0=$ time tolerance factor, $n=$ rate constant (depending on diffusion, etc.). The same type of deviation can be recognized in Powers's (1917) results where the slope of the "velocity of fatality" $\left(\frac{1}{\text{survival time}}\right)$ curve falls off above a certain point. Finally, Peters (1936), who regards the deviation as due to saturation of the tissues of the organism, proposes the correction

$$(c-c_s)\,t=W.$$

The value c_s is quite different from c_0; it is not a constant but represents the excess of concentration over the saturation of the tissues.

These short exposure deviations seem only to have been noted in experiments where the time measured was the survival of the organism in a toxic medium. It is, therefore, possible that the theoretical lag at infinite concentrations may be actually the time required for biological response to become manifest. If this is true, we should not expect the

deviation in the results of fumigation experiments since the time measured is merely exposure and not the period before death.

Support is given to this view by results of Peters (1936) (though he interprets them otherwise). He gives the results of some ethylene oxide experiments carried on until complete kills were obtained (Sofortwirkung) and others in which mortality was estimated 1·5 days after the exposures (Spätwirkung). The time-concentration products in the first series were found to be higher in the short than the long experiments, while in the second series no such difference was evident.

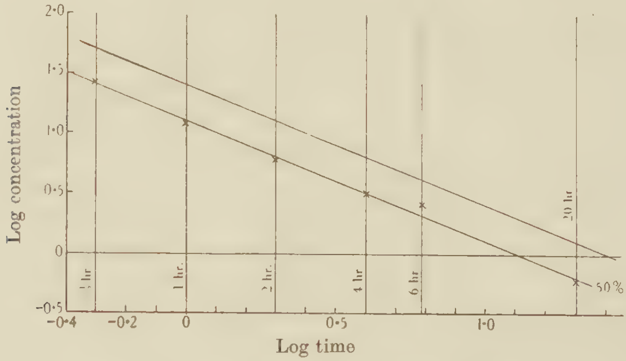

Fig. 8. Dose-time relations with *Calandra oryzae*.

Assuming that the present results, like the "Spätwirkung" series of Peters, do not show any short exposure deviations, they may be fitted with the simple empirical formula:

$$c^n t = W,$$

which gives a linear relation when converted to logarithms:

$$n \log c + \log t = K.$$

The slope of this line is given by n. Agreement with Haber's formula will be indicated by values about unity while the possibility of threshold concentrations are met by values of n greater than one.

To apply this formula to the present work, a constant mortality must be chosen. The 50 % kill point has been taken because of reliability and general use. But since the regression lines do not show any constant change of slope, a similar result should be obtained with any other predetermined mortality. The figures have been treated statistically to determine if they will fit the equations within the limits to be expected from the individual regression lines.

The agreement between the observed results and the proposed formula can be judged from Figs. 8–11. Table XIV gives the equation constants for each insect and also statistical details.

A satisfactory fit was obtained for every insect except *Calandra granaria*. Here the high value of χ^2 suggests some inconsistency which is probably due to either the $\frac{1}{2}$ hr. or 20 hr. point (see Fig. 9). The values

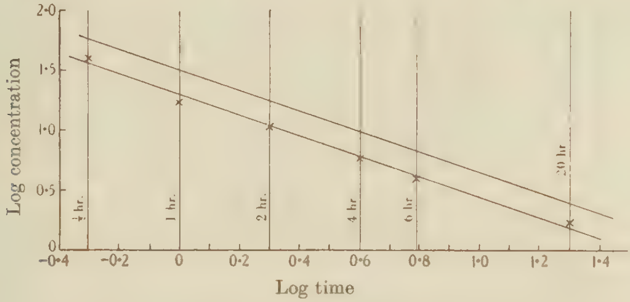

Fig. 9. Dose-time relations with *Calandra granaria*.

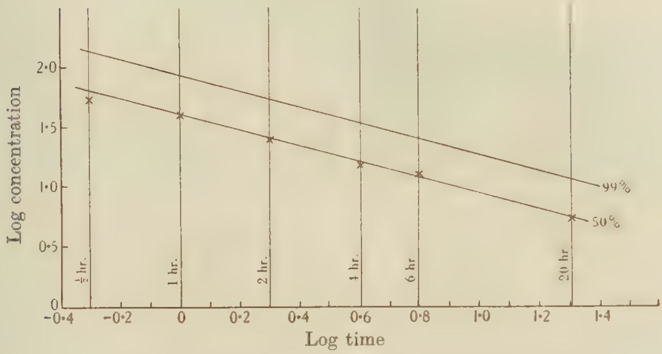

Fig. 10. Dose-time relations with *Tribolium castaneum*.

of P are not given since their precise significance is uncertain. They might reflect heterogeneity of material or measurement errors as well as departure from the proposed equations.

In general it is claimed that the formula $c^n t = W$ provides a satisfactory method of expressing concentration-time curves since no consistent deviations were shown by the four test insects.

A practical implication of the acceptance of this formula is the fact that only two exposure times need be investigated to solve the equation

and give the whole curve. This should economize the work of evaluating fumigants over a range of exposures.

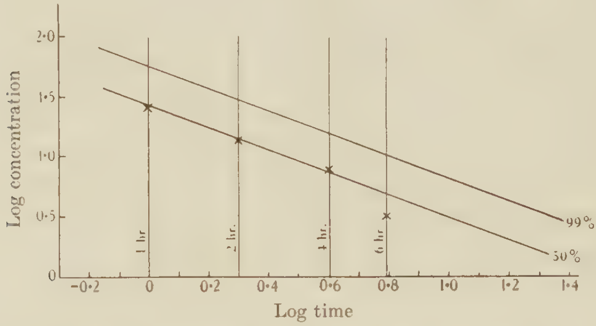

Fig. 11. Dose-time relations with *Cimex lectularius*.

Table XIV

Time-concentration relations

Insect	Regression equation $c^n t = W$	Standard error of n	Degrees of freedom	χ^2
Tribolium castaneum	$c^{1\cdot51}t = 40\cdot6$	0·033	5	11·149*
Calandra granaria	$c^{1\cdot18}t = 19\cdot3$	0·026	4	18·454
C. oryzae	$c^{1\cdot00}t = 12\cdot6$	0·031	4	5·882
Cimex lectularius	$c^{1\cdot05}t = 26\cdot8$	0·082	2	6·527

* Major fraction of this value due to one discrepancy which, when tested exactly, was found not to be significant. Hence no departure from calculated line for *Tribolium*.

(3) *Expression of toxicity and resistance*

(*a*) *Units.*

Examination of the literature concerning insect fumigants reveals a great lack of uniformity in expression of dose or concentration. Without considering the numerous ways of giving dosages there are four main ways of expressing concentrations attained. They may be mentioned as percentage by volume or by weight or as milligrams per litre, either under experimental conditions, or reduced to normal temperature and pressure. The percentages have the advantage that they denote a constant air-fumigant mixture, whatever the temperature, but the "milligram per litre" figures are easier to interpret into practical doses. As a compromise mg./litre at N.T.P. has been employed in this work, since it combines both the above advantages.

Time can be expressed conveniently in hours.

(*b*) *Criteria of resistance.*

Attempts to compare toxicities or resistances from published data are complicated by the variety of exposure times chosen. Usually only one period is chosen, and this may be anything from $\frac{1}{2}$ to 48 hr. In order roughly to compare such heterogeneous data and to find a simple criterion of resistance, the general nature of time-concentration relations must be considered. In the previous section, we have found the equation $c^n t = W$, where $n = 1$ to 2 satisfactory. As an approximation the product $c.t$ may be taken as constant ($n \simeq 1$) and this provides a simple measure of resistance. It can be used to assess the effect of a practical fumigation where the concentration is variable. (If the course of the variation be plotted graphically the $c.t$ constant can be found by integrating the area under the curve: Peters & Ganter, 1935*a*.)

Unfortunately, the value of n is sometimes considerably greater than one (*Tribolium*—ethylene oxide = 1·5 (Table XIV); *Drosophila*—hydrogen cyanide = 1·8 (Bliss & Broadbent, 1935)). As a result the product $c.t$ increases steadily with longer exposures. Therefore, the value n must be mentioned in expressing resistance because some insects are relatively more resistant to long exposures than others.

Bearing in mind that the criterion should be brief and readily interpretable, it is proposed that it should consist of the 50 and 99% lethal concentrations for a 5 hr. exposure together with the value of n. From these figures the whole curves can be calculated if necessary. Table XV gives the relevant criteria for the four test insects to ethylene oxide at 25° C.

Table XV

Criteria of resistance to ethylene oxide

Insect	5 hr. 50% lethal concentration (mg./l. N.T.P.)	5 hr. 99% lethal concentration (mg./l. N.T.P.)	Value of n
Calandra oryzae	2·9	4·1	$1{\cdot}00 \pm 0{\cdot}031$
C. granaria	5·5	8·4	$1{\cdot}18 \pm 0{\cdot}026$
Cimex lectularius	6·6	12·3	$1{\cdot}05 \pm 0{\cdot}082$
Tribolium castaneum	16·6	27·0	$1{\cdot}51 \pm 0{\cdot}033$

Summary

1. The orders of resistance of four different species of insects are shown to be different towards hydrogen cyanide, ethylene oxide and sulphur dioxide.

2. The different circumstances to be considered in fumigation research are surveyed with a view to ensuring reliable results. They can be grouped as:

(*a*) Factors which may influence absorption of poison.

(*b*) Factors which may influence toxic processes and degree of poisoning.

3. These factors are assessed and each aspect of technique is described with an estimate of the extent to which it has been controlled.

4. The arrangement of experiments to provide as much information as possible is described and the limits of accuracy defined.

5. Details of the rearing and treatment of insects are given.

6. Difficulties of estimation of mortality are considered and a relation between severity of fumigation and incidence of death or recovery from stupefaction is demonstrated.

7. The dose-kill data are treated by Bliss's Method of Probits and tables of statistics for each set of data are appended.

8. Various time-concentration relations are considered, and from these the empirical formula $c^n t = W$ has been adopted. A statistical analysis shows no serious deviation from this relationship.

9. The need for uniformity in expression of toxicity is stressed, and the following criterion of resistance proposed: "The 5-hr. concentrations (mg./l. N.T.P.) for 50 and 99% kill and the value of n".

10. The values of the criteria for the four species used (*Calandra granaria*, *C. oryzae*, *Tribolium castaneum* and *Cimex lectularius*) are given.

I should like to express here my deep gratitude to Prof. Munro for encouraging me to undertake this work and also to Dr Bovingdon, Dr Lubatti and Dr Bartlett for their technical advice.

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STUDIES ON AMERICAN FOUL BROOD OF BEES

II. THE GERMINATION OF THE ENDOSPORES OF *BACILLUS LARVAE* IN MEDIA CONTAINING EMBRYONIC TISSUES

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WITH AN APPENDIX

EXPECTED ERRORS IN DILUTING BACTERIAL SUSPENSIONS

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DURING the past few years the use of the tissues of the developing chicken embryo as a bacteriologically sterile culture medium for many animal and human viruses has greatly increased. Two general techniques have been developed, one involving the direct inoculation of the chorioallantoic membrane of the developing embryo (Burnet, 1936), and the other the preparation of an embryo "brei" by mincing whole embryos under aseptic conditions (Dochez *et al.* 1936; Li & Rivers, 1930; Magill & Francis, 1936; Nelson, 1936).

Bacillus larvae, the organism responsible for American foul brood, requires rather complex media for the germination of its endospores and multiplication of the vegetative cells arising therefrom (Lochhead, 1928; Maassen, 1919; Sturtevant, 1924, 1932; Tarr, 1937; White, 1907, 1920). This is hardly surprising since many of the bacteria pathogenic for man and animals are likewise fastidious with respect to their nutrient requirements, a large inoculum of such organisms frequently being necessary to initiate growth even on "rich" media (Knight, 1936). Sturtevant (1932) showed that, normally, several million spores of *B. larvae* are required to produce vegetative growth on a complex egg yolk carrot extract medium, and that the presence of reducing sugar (glucose) in this medium in concentrations slightly in excess of 2·5% either markedly inhibited, or entirely suppressed, the germination of the endospores and the multiplication of the vegetative cells of this species. It

occurred to the writer that the tissues of the developing chicken embryo might provide a nutrient substrate for *B. larvae* more favourable than those hitherto employed, and that, if this proved to be the case, the sensitivity of this organism to reducing sugars in various concentrations could be tested conveniently in this medium. The results of experiments designed to test these points are recorded below.

Experimental

The spore suspensions of *B. larvae* employed were prepared directly from the ropy remains of larvae dead of American foul brood under aseptic conditions, and not from pure cultures of this organism as in previous experiments (Tarr, 1937). The ropy larvae, which contained *B. larvae* spores in apparently pure culture, were ground in distilled water, the coarse debris being removed from the resulting suspension by passing it through two layers of Whatman No. 1 filter paper. The spores in the filtrate were washed by centrifuging and were suspended in water. The suspensions were stored at about 2° C. and were used within 10 days of preparation. The number of spores per ml. in such suspensions was estimated by means of a Thoma haemocytometer slide, a large number of microscopic fields being counted in each instance. The standard error for each total count was kindly estimated by Mr Cochran. Serial dilutions were prepared from these suspensions, immediately prior to use, by mixing the suspension thoroughly, removing 1 ml. with a sterile pipette (accurate within approximately $\pm 2\%$), adding this volume to 9 ml. of sterile distilled water, and repeating the procedure until the desired set of dilutions was obtained. This dilution method is subject to certain inaccuracies which are discussed in an appendix to this paper (see p. 640). In the experiments to be described, in stating the number of spores introduced with each inoculum due allowance has been made for both the error due to the method of counting and to that of making the dilutions.[1] There is the possibility that a certain proportion of the *B. larvae* spores in such suspensions are "non-viable" even under ideal conditions but, unfortunately, there is no reliable method of determining whether such spores are present.

The egg yolk and beef digest brood filtrate agar media were prepared as previously described (Tarr, 1936). Embryo brei medium was prepared usually from 14-day-old chicken embryos, but occasionally from 12-day-old embryos, by mincing them in Tyrode solution (pH approximately 7·2), following, in general, the method of Li & Rivers (1930). 20–25 ml.

[1] I am indebted to Mr Cochran for determining these errors.

of Tyrode solution was used for each embryo. Immediately after preparation portions of the brei were pipetted into sterile test tubes, using wide mouth glass pipettes. There is always danger that a medium prepared in this manner may be contaminated, and, unfortunately, it cannot be tested for sterility by the customary method of incubating prior to inoculation because the developing embryonic tissues die fairly rapidly, especially at higher temperatures of incubation. However, experience has shown that if the medium is prepared as quickly as possible, with all reasonable aseptic precautions, it is rarely contaminated. It has usually proved easy to differentiate *B. larvae* from the contaminating organism, and to isolate this bacillus in pure culture. In a few instances *B. larvae* was cultivated on the chorioallantoic membrane of the developing embryo, the technique described by Burnet (1936) being followed.

The various media were inoculated with aqueous suspensions of *B. larvae* spores and incubated at approximately 35° C. When a microscopical examination of a given culture, made by streaking a portion of the medium on a slide with a small amount of a saturated aqueous solution of nigrosine, indicated positive growth of *B. larvae*, the presence of this organism in pure culture was confirmed by the following tests. Transfers were made on to beef digest brood filtrate agar slopes, and the characteristic cultural and morphological features of the resulting cultures were recorded. All cultures were tested for nitrite since Lochhead (1928, 1937) has shown this test is strongly positive when *B. larvae* has grown in many culture media even without added nitrate. In the writer's experiments it was found that nitrite was not produced when *B. larvae* grew in embryo brei medium unless nitrate was added to the medium. Thus, a positive nitrite test resulted when one or two drops of a sterile 5% solution of potassium nitrate were added to an embryo brei culture in which *B. larvae* was growing and the medium was reincubated for 16–24 hr. at 35° C.

Exp. 1. A suspension containing $141 \times 10^6 \pm 2{\cdot}8\%$ spores of *B. larvae* per ml. was used. Embryo brei was tubed in 5 ml. portions in $6 \times \frac{5}{8}$ in. test-tubes, and egg-yolk and beef digest brood filtrate agar were sloped in 6 ml. amounts in similar tubes. These media were inoculated with 1 ml. portions of the various dilutions of the above spore suspensions, the agar cultures being sealed with paraffin wax in order to prevent undue drying of the media on prolonged incubation. The chorioallantoic membranes of 10–12-day-old chicken embryos were inoculated with 0·01 ml. of spore suspensions using an Agla micrometer syringe, a slight rupture being made in the membrane if this had not occurred on separation of shell and chorioallantoic membranes. All cultures were incubated at 35° C., periodical microscopical examinations being made

of the embryo brei and chorioallantoic membrane cultures to ascertain whether or not growth had occurred. A maximum period of 30 days was given for incubations. Although it is possible that spores may remain dormant for longer periods this length of time was considered adequate for the experiments recorded. The results of this experiment are given in Table I, the number of tubes of medium inoculated from each dilution being recorded in brackets beside the number of tubes showing positive growth within the 30-day period.

Table I

The ability of B. larvae *spores to germinate in different media*

No. of spores of *B. larvae* introduced (standard error due to counting and dilution factors given)	No. of cultures of those inoculated showing growth of *B. larvae* (no. of tubes inoculated in brackets): Egg-yolk agar	Beef digest brood filtrate agar	Embryo brei	Chorioallantoic membrane
$141 \times 10^6 \pm 2{\cdot}8\%$	(6) 3	(6) 5	(2) 2	—
$141 \times 10^5 \pm 3{\cdot}0\%$	(6) 0	(6) 3	(2) 2	—
$141 \times 10^4 \pm 3{\cdot}1\%$	(6) 0	(6) 0	(2) 2	(1) 1
$141 \times 10^3 \pm 3{\cdot}3\%$	(6) 0	(6) 0	(2) 2	(1) 1
$141 \times 10^2 \pm 3{\cdot}5\%$	(6) 0	(6) 0	(2) 2*	(1) 1
$141 \times 10^1 \pm 4{\cdot}4\%$	(6) 0	(6) 0	(2) 2	(1) 1
$141 \pm 9{\cdot}2\%$	(6) 0	(6) 0	(5) 4	(1) 1
$14 \pm 26{\cdot}9\%$	—	—	(3) 0	—
1·4	—	—	(3) 0†	—
0	—	—	(3) 0	—

* One contaminated by a coccus but *B. larvae* isolated in pure culture.
† One contaminated by a torula; no sign of *B. larvae*.

It is evident from these results that both the embryo brei medium and chorioallantoic membrane are much more favourable substrates for the germination of *B. larvae* spores than either of the agar media. Only the limited number of experiments recorded in Table I have been made with the chorioallantoic membrane technique, and it would be interesting to make a large number of inoculations using embryos of different ages and various doses of spores. Further experiments recorded below have all been made using embryo brei medium.

Exp. 2. A suspension containing $105 \times 10^6 \pm 3{\cdot}2\%$ *B. larvae* spores per ml. was used. Dilutions were prepared from this suspension in the usual manner and 1 ml. portions were inoculated into tubes containing 5 ml. of embryo brei medium each. Two different series of experiments were made using various numbers of tubes of embryo brei prepared from different embryos and various doses of spores. The results of this experiment are set out in Table II.

From the limited data so far obtained it seems inadvisable to attempt to make any prediction regarding the number of *B. larvae* spores which can be expected to germinate on embryo medium. It would be interesting to make a large series of inoculations using different batches of embryo

brei and inocula of spores ranging from approximately 10,000 to ten from various spore suspensions. It appears, from the relatively small number of experiments made, that 1000 spores will usually cause growth while ten spores are extremely unlikely to do so.

Table II

Germination of B. larvae *spores in embryo brei medium*

No. of spores of *B. larvae* introduced (standard error due to counting and dilution factors given)	No. of tubes inoculated	No. of tubes showing growth of *B. larvae*
	A	
10,500 ± 3·9%	3	3*
1,050 ± 5·0%	3	3
105 ±10·6%	3	1
10·5 ±31·1%	3	0†
1	3	0
	B	
1,050 ± 5·0%	8	7
105 ±10·6%	8	3
10·5 ±31·1%	8	0‡

* One contaminated but *B. larvae* isolated in pure culture.
† One contaminated by a coccus, no sign of *B. larvae*.
‡ One contaminated by an aerobic bacillus, no sign of *B. larvae*.

In further experiments the effect of added available nitrogen, in the form of beef digest broth, and reducing sugars on the germination of *B. larvae* spores in embryo brei have been investigated.

Exp. 3. Tubes containing 4 ml. of embryo brei and 1 ml. of Hartley's beef digest broth were prepared, and these were inoculated with various dilutions of the spore suspension used in Exp. 2. The results obtained are given in Table III.

Table III

The effect of beef digest broth on germination of B. larvae *spores in embryo brei medium*

No. of spores of *B. larvae* introduced (standard error due to counting and dilution factors given)	No. of tubes showing positive growth of *B. larvae* out of four inoculated
$105 \times 10^4 \pm 3{\cdot}5\%$	4
$105 \times 10^3 \pm 3{\cdot}6\%$	4
$105 \times 10^2 \pm 3{\cdot}9\%$	4
$105 \times 10^1 \pm 5{\cdot}0\%$	3
105 ±10·6%	0*
10·5 ±31·1%	0

* One contaminated by a coccus.

From the results obtained it appears as if beef digest broth hinders rather than promotes germination of *B. larvae* spores in embryo brei medium.

Exp. 4. Embryo brei medium was prepared with added beef digest broth (this was added before it was found that added nitrogen appears to hinder slightly the germination of *B. larvae* spores in the embryo medium). Various amounts of 25 or 50% aqueous solutions of an equimolecular mixture of fructose and glucose (sterilized by autoclaving) were added to the brei medium so that concentrations of reducing sugar in the final media varied from 0 to 12·5%. Each tube was inoculated with 0·1 ml. of a dilution of the spore suspension used in Exp. 2, $105 \times 10^3 \pm 3{\cdot}6\%$ spores being introduced. In Table IV the set up and results of this experiment are given. No apparent deleterious effect of even 12·5% reducing sugar on germination of *B. larvae* spores was observed. In all cases positive growth of *B. larvae* took place within 3 days of inoculation, and in most instances within 1 or 2 days. This experiment is open to criticism on the grounds that the embryonic tissues might destroy the reducing sugar, thus rendering the medium suitable for the growth of *B. larvae*; while this point has not been checked by measuring the amount of reducing sugar present in the media after *B. larvae* has commenced to grow, it is extremely doubtful if the amount of tissue present would destroy more than a fraction of a 12·5% solution of reducing sugar. The *p*H of the media after the growth of *B. larvae* had occurred was about 4·5; and those containing the higher concentrations of reducing sugars were still distinctly sweet to the taste.

Table IV

Germination of the endospores of B. larvae *in embryo brei medium containing reducing sugars*

Composition of medium							
Embryo brei ml.	Glucose-fructose solution ml.		Beef digest broth ml.	Spore suspension ml.	H_2O ml.	Final concentration of reducing sugar %	No. of cultures showing growth (quadruplicates)
3	0	25%	0·9	0·1	1·0	0	4
3	0·2	25%	0·9	0·1	0·8	1	4
3	0·4	25%	0·9	0·1	0·6	2	4
3	0·6	25%	0·9	0·1	0·4	3	4
3	0·8	25%	0·9	0·1	0·2	4	4
3	1·0	25%	0·9	0·1	0·0	5	4
2·5	0·5	50%	0·4	0·1	0·5	6·25	4
2·5	0·75	50%	0·4	0·1	0·25	7·38	4
2·5	1·0	50%	0·4	0·1	0·0	12·5	4

Discussion

Embryo brei prepared from chicken embryos, and the chorioallantoic membrane of the developing egg, are by far the most favourable media yet found for the germination of the endospores of *Bacillus larvae* and, in these substrates, the vegetative cells of this organism multiply rapidly. Added available nitrogen in the form of beef digest broth to the embryo brei medium tends to inhibit rather than favour the germination of the spores.

It is of interest that germination of the spores and multiplication of the vegetative cells of *B. larvae* took place in the presence of concentrations of reducing sugars as high as 12·5% in embryo brei medium. Higher concentrations have not yet been tried. This finding is rather unexpected in view of the fact that Sturtevant (1924) found that relatively low concentrations of glucose (2 3%) either seriously impaired, or totally inhibited, spore germination or vegetative cell multiplication of *B. larvae* on egg-yolk agar medium. He suggested that the reason that *B. larvae* normally attacks larvae only after the cells containing them have been sealed is because, at this stage, the reducing sugar in them has fallen to such a low level that it ceases to inhibit the spores of this organism from germinating. This attempted correlation of the events occurring in a culture medium with those taking place in the larva itself is open to the criticism that the factors which tend to inhibit growth of *B. larvae* in a culture medium are totally different from those operating in a living organism. At present, it cannot be stated definitely that reducing sugar concentration plays any role in determining at what stage in larval life American foul brood develops. Much remains to be learned regarding the "age incidence" of American foul brood, especially in view of the body of evidence which has recently been accumulated (Tarr, 1937, 1938) relating to the probable part played by the adult bee in carrying the disease.

Summary

The tissues of the developing chicken embryo form a more suitable substrate for the germination of the endospores of *Bacillus larvae* than any medium so far described.

The addition of beef digest broth to a medium of minced chicken embryo hinders rather than promotes the germination of *B. larvae* spores.

Concentrations of reducing sugars as high as 12·5% cause no apparent hinderance in the germination of *B. larvae* spores in embryo medium.

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APPENDIX

Expected errors in diluting bacterial suspensions

By W. G. Cochran

From a solution containing a known number of spores or vegetative cells per unit volume, higher dilutions are commonly prepared by extracting a small measured volume in a pipette (usually 1 ml.) and adding this to a larger volume of water (usually 9 or 99 ml.). It is realized that if a number of, e.g. $\frac{1}{100}$, dilutions are prepared from the same solution, these dilutions will not all contain exactly $\frac{1}{100}$ of the original number of, e.g. spores, per unit volume. With skilful work, the two principal sources of variation appear to be (1) the sampling variation involved in taking out a small measured volume by pipette; (2) errors in the volume actually removed by pipette. A 1 ml. pipette will not always remove exactly 1 ml. and, indeed, most manufacturers specify the limits of error of their pipettes.

Assuming that the solution is thoroughly mixed at each stage of the dilution before removing a small volume by pipette, and that there is no clumping of the spores or vegetative cells on mixing, the sampling

errors in the number of spores removed per unit volume will follow a Poisson series distribution and are known if the mean number of spores per unit of the original volume is known. An allowance may also be made for the measuring errors of the pipette, according to its standard of accuracy. This allowance may also be made to include errors in the measurement of the volume of water to which the solution in the pipette is added on making the dilution, though it seems likely that these errors will be relatively negligible. Thus, from theoretical considerations alone, we can assign limits of accuracy to the number of spores or bacterial cells in the volume of solution which is being used in any dilution experiment. These limits are to be regarded as attainable with competent work.

Table V

Variation due to dilution and pipetting

No. of spores etc.	Standard error %	5% limits Lower %	5% limits Upper %	No. of spores etc.	Standard error %	5% limits Lower %	5% limits Upper %
10^6	1·00	98·0	102·0	120	9·18	82·8	118·8
10^5	1·05	97·9	102·1	100	10·1	80·8	120·8
2×10^4	1·22	97·6	102·4	90	10·6	79·8	121·7
10^4	1·41	97·2	102·8	80	11·2	78·6	123·1
4×10^3	1·87	96·3	103·7	70	12·0	77·2	124·8
2×10^3	2·45	95·1	104·9	60	13·0	75·4	126·9
$1{\cdot}4\times10^3$	2·85	94·3	105·7	50	14·2	73	130
10^3	3·32	93·4	106·6	45	14·9	72	131
800	3·67	92·9	107·3	40	15·8	70	133
600	4·20	91·9	108·4	35	16·9	68	136
500	4·58	91·2	109·2	30	18·3	65	139
400	5·10	90·2	110·3	25	20·0	62	143
300	5·86	88·8	111·8	20	22·4	57	149
200	7·14	86·6	114·4	15	25·8	49	157
150	8·23	84·5	116·7	10	31·6	38	172

Note. The standard error due to pipetting was assumed to be 1% in computing the above table.

Table V shows, for a wide range of concentrations, the standard error percentage of the number of spores or vegetative cells and the lower and upper limits (also as percentages) within which the number will lie in 95% of cases. It must be stressed that these errors refer to the number of spores in that volume of solution which is being used for experimental purposes. To take an example, suppose that a solution containing $1{\cdot}5\times10^5$ spores per ml. is diluted to $\frac{1}{1000}$ and that 1 ml. of the dilute solution is removed for experimental purposes, e.g. the inoculation of an experimental medium to ascertain the number of spores which will initiate growth. The expected number of spores in 1 ml. of the dilute solution is 150. Reference to the diagram shows that the

standard error of this figure is 8·2 % and that the 95 % lower and upper limits are 84·5 and 116·7 % respectively. Thus the number of spores in the ml. of solution is fairly certain to lie between 84·5 and 114·4 % of 150, i.e. between 127 and 175. If, however, only $\frac{1}{10}$ ml. of this dilution is being used, the reading must be taken at the point 15 and not at 150, since there are only about 15 spores in the volume which is actually being used. The standard error at this point is 25·8 % and the limits of accuracy are 7·35 and 23·55, or, taking the widest *integral* range within these limits, between 8 and 23.

With numbers of spores below 50, it is not possible to give 5 % limits which are at all exact, since the number of spores must be a whole number with a finite probability. For instance, the exact probability of getting numbers of spores outside the limits 8 and 23 in the above example is 3·7 % instead of 5 %, while the probability of getting numbers outside the limits 7 and 22 is 7 %.

The entries in Table V are not evenly spaced, as the standard error and limits change more rapidly as the dilutions become higher. The range is, however, sufficiently well covered to give reasonably accurate limits for values intermediate between those shown in Table V. Proportional parts will not, however, give a good interpolation in the lower part of the table. If accurate interpolation is required, proportional parts should be used on the reciprocals of the square roots of the numbers of spores.

To construct Table V, some assumption was necessary about the error of pipetting, though this is important only at low dilutions. The standard error was assumed to be 1 %. Thus, in all but 5 % of cases, the volume extracted by the pipette was assumed correct to within 2 %. This probably represents the limits of error of good pipettes. The table can be constructed for any given standard of accuracy in pipetting; however, to find the limits of variation in the number of spores with pipetting which is correct to say, 4 %, it is sufficiently accurate for most purposes to regard the 5 % limits of accuracy as ±4 % until the dilution is sufficiently high that the limits in the table rise above 4 % and, thereafter, to use the limits in the table. In this connexion it should be noted that after, e.g. three dilutions using pipettes with a 1 % standard error, the standard error of the dilute solution due to pipetting is, not 1 %, but $\sqrt{3}\,\% = 1{\cdot}73\,\%$; and if the dilution is still so low that sampling errors are negligible, the 5 % limits of accuracy are ±3·5 %.

Table V covers the range from 10 to 10^6 units. Below 10 units a single sample is extremely variable. Above 10^6 units the whole error is due to pipetting.

The number in the original suspension will not usually be known exactly, but will itself be estimated with a known standard error. In this case the standard error of a dilute suspension is found by adding the square of the standard error in Table V to the square of the original standard error, and taking the square root. The standard errors given in Tables I, II and III were obtained in this way. As an example, we will derive the standard error of the dilution 141×10^1 in Table I. The original standard error is 2·8 %, and from the table the standard error due to dilution, with a 1 % pipette standard error, is also about 2·8 %. Since, however, there have been five successive dilutions, the pipetting standard error is $\sqrt{5}$ %. Thus the standard of the dilute suspension is

$$\sqrt{\{(2{\cdot}8)^2 + (2{\cdot}8)^2 + (5 - 1)\}} = 4{\cdot}4\ \%.$$

If the additional error due to repeated pipetting were ignored in the above calculation, as previously suggested, the result would be 4·0 %.

(*Received* 4 *January* 1938)

PROCEEDINGS OF THE ASSOCIATION OF APPLIED BIOLOGISTS

Ordinary Meeting of the Association held at 2.30 p.m. on Friday, 18 March 1938, at the Imperial College of Science and Technology, London, the President, Mr C. T. Gimingham, in the Chair.

Discussion on the Use of Chemical Weedkillers

The following papers were read:

I. Chemical weedkillers in relation to horticulture. By M. A. H. Tincker, M.A., D.Sc.

II. Some factors influencing the agricultural use of chemical weedkillers. By R. K. MacDowall, Dipl.R.T.C., A.M.I.Chem.E.

III. The relative toxicity of chemical weedkillers. By G. E. Blackman, M.A.

IV. The control of weeds in lawns and fine turf. By R. B. Dawson, M.Sc., F.L.S.

V. Chlorate weedkillers. By O. Owen, M.Sc., Ph.D., A.I.C.

I. CHEMICAL WEEDKILLERS IN RELATION TO HORTICULTURE

By M. A. H. Tincker, M.A., D.Sc.

Keeper of the Laboratory, Royal Horticultural Society, Wisley, Ripley, Surrey

In 1934 the experimental work carried out at Wisley was reported, together with a brief review of the literature. In the ensuing interval further experience has been gained, chiefly from advisory work, of the relative success or failure of several chemical weedkillers when used in frequent conjunction with the usual routine carried out in different departments of the industry. It will be convenient to consider these departmental aspects separately.

Orchards

(*a*) *Cultivated.* Only with the greatest care can sodium chlorate be used to eradicate the arable weeds in cultivated orchards. Nettles (*Urtica dioica* and *U. urens*), groundsel (*Senecio vulgaris*) and annual meadowgrass (*Poa annua*), fat-hen (*Chenopodium album*), chickweeds (*Cerastium vulgatum* and *Stellaria media*), chamomiles (*Anthemis arvensis* and *A. Cotula*), and other weeds can be killed quickly in early autumn by a $2\frac{1}{2}$–3% spray of sodium chlorate. This is a most inconvenient time, as the fruit harvest is then in progress. In early spring smaller weeds can be eradicated similarly. Approximately 1 gal. of solution to 10 sq. yd. suffices for a

fairly thick covering of weeds; for smaller weeds a lighter application may suffice. The spray solutions must be applied carefully and the ground must not be soaked, especially where surface rooting of the fruit trees has been encouraged by previous manurial treatments. Where smaller fruit has been interplanted between standard top fruits further care in spraying is necessary. The tar oil washes used in early spring check the growth of many common weeds, but recovery usually takes place later.

Cyanamide is useful on soils with a low nitrogen content. It can be used for several consecutive seasons; by application to the weeds when they are wet and by the subsequent bruising and burial by mechanical or other cultivation processes, accelerated decomposition to humus is facilitated and the tilth and soil composition is thus improved. Cyanamide fails to eradicate couch grass (*Agropyron repens*) without repeated cultivation.

Arsenical weedkillers are unsuitable for continued orchard use; their toxicity is low and the arsenic tends to remain in the soil, though Crofts (1933) found no serious soil damage from acid arsenical sprays.

There appears to be little or no readily available information concerning the use of sulphuric acid in fruit orchards. Only a small amount of damage to the soil, due to decomposition of the carbonates, results from application of sulphuric acid used to kill annual weeds under arable field conditions. The method is worthy of further tests.

(*b*) *Grass orchards*. Where there is a grass covering it is somewhat safer to use sodium chlorate as a weedkiller to eradicate in this case nettles (*Urtica* spp.), ragwort (*Senecio Jacobaea*), and thistles (*Cirsium acaule*, *C. arvense*, and *C. lanceolatum*). Every care must be taken to apply the spray of the solution of the weedkiller to the weeds only and to prevent drenching or soaking the ground. If only light applications be given carefully to the fully developed foliage of the weeds the method is successful and no damage results to the trees.

It is seen that weed eradication in orchards cannot be accomplished by chemical weedkillers without much care.

Market gardens and vegetable growing

The poisonous nature of the arsenical weedkillers, and the likelihood of food contamination, generally precludes their use. All the vegetables tested, as yet, are very sensitive to damage from chlorates; so that during the growing periods and for some time previously, determined by the soil conditions as they influence drainage and percolation, the application of chlorates as weedkillers is not practicable. Korsmo (1932) tested numerous vegetables to ascertain their reaction to 4 % solutions of sulphuric acid. He reported the following results:

(*a*) Undamaged by the acid: cabbage, cauliflower, leek, onion, and shallot.

(*b*) Damaged but recovered: peas, lettuce, swede, salsify, purslane, dill, thyme.

(*c*) Damaged as severely as the weeds: carrot, radish, beetroot, celery, parsnip, broad bean, dwarf bean, French bean, parsley.

Thus, the use of sulphuric acid is very restricted in market garden work.

There can be no question of the value of cyanamide in the previous preparation of weedy ground for vegetable culture, since the improved quality of vegetables when

grown with adequate humus in the soil is undoubted; this is additional to the manurial value of the mineral constituents of the fertilizer.

It appears, then, that cultivation methods must still be relied upon for weed eradication in this section.

Lawns and grass

The Bingley methods, advocated by Dawson & Evans (1931 *a*), of eradicating weeds by a mixture of ammonium sulphate and ferrous sulphate have, in general, given satisfactory results on soils of the Bagshot sands, Folkestone beds, London clays and other non-calcareous soils. For ecological details of closely cut turf so treated Blackman's paper (1932*a*) should be consulted. But the eradication of weeds from lawns on chalk soils, such as the downlands, is more difficult. To build up a lime-free surface layer is often expensive, and the repeated application of sulphate of ammonia and iron, followed by repeated cuttings of the grass, frequently results in thin poor grass if little or no other fertilizers be given for several seasons. Moreover, the weeds do not so readily disappear on these soils by such methods. The "acid theory" is inapplicable where the surface layers contain much chalk, but a chalk-free layer is not unknown above pure chalk.

How does *Brachypodium pinnatum* compete against the chalkland weeds? Perhaps an ecological study might reveal interesting data although this species is not itself likely to be useful.

In America the use of weak solutions of chlorate to eradicate such weeds as *Glechoma hederacea* has been recommended by Walton (1929). At $2\frac{1}{2}\%$, grass may be severely checked, as in our tests when discoloration was apparent, but ultimate recovery took place. This method may be of value in conjunction with later resowing of the grass. A solution of 1% sodium chlorate will injure certain weeds of lawns and cause less damage to the grass, but the method has few real advantages to offer in comparison with a "spot" treatment with sulphates of ammonia and iron.

Hard tennis courts

Hard tennis courts are sometimes laid down on soils from which the root systems of weeds have not been adequately removed. Bindweed (*Convolvulus arvensis*) may spread very rapidly in the moist coarse ashes frequently used below the granite chips. In one case thousands of shoots appeared through the granite type of surface within a few days. By permitting the early stages of leaf development the use of a 5% solution of sodium chlorate proved highly effective when applied at 2 gal. for each 10 sq. yd., and no subsequent trouble with this weed was reported. Subsequent watering was carried out to wash the remaining chemical into the lower layers of the court. Unless this is done the granite chips dry at a rate slower than usual, and the danger of fire remains. (Calcium chlorate decreases the rate of drying.)

Paths and drives

Generally, the application of sodium chlorate at 5% (or if no grass is present, at $2\frac{1}{2}\%$) is effective for one season. Arsenical weedkillers, such as 2% sodium arsenite, often have a more persistent effect, lasting for 2 or 3 years at a similar rate of application. It has been observed fairly frequently, in damp localities, that subsequent invasion by mosses takes place readily some months after chlorate has been used.

There is a fire danger arising from use of chlorates on drives where flints may cause a spark as motor cars pass but, fortunately, no cases have been reported of serious damage in this country.

Neglected ground

Sulphuric acid at 10% has sometimes failed to kill large tufts of certain grasses including *Dactylis glomerata*, *Holcus lanatus*, and *Alopecurus pratensis*, but has generally proved effective when applied to *small* broad-leaved weeds.

If there are *many* thick tussocks of well-established grass it has been necessary to use a 10% solution of sodium chlorate to effect complete killing at 1 gal. to 10 sq. yd. Vegetation so killed may be raked together and burnt, but labour is required to clear the debris. The fallow period for soil recovery varies inversely as the rate of the drainage; a short period of two months was sufficient on certain Bagshot sands in winter to permit of sowing lupins and lettuce. On heavier soils, five or six winter months should elapse before any seed sowing or planting can be safely undertaken.

Mention has already been made of the value of cyanamide in conjunction with cultural methods and smother crops for eradicating couch grass and the subsequent rotting of the vegetable matter. The tests made by Scarlett (1937) at Musselburgh and elsewhere, show that a period of 12–15 months is required for this process; it appears unlikely that this period can be materially reduced advantageously.

Acid arsenical weedkillers, which combine the application of sulphuric acid and arsenite, have been reported by Crofts (1933) to give satisfactory results in the eradication under Californian conditions, of certain very troublesome weeds, such as *Convolvulus arvensis* and *Centaurea repens*. For the reaction to sulphuric acid alone, the lists of species drawn up by Aslander (1927) should be consulted, where it will be seen that the leaves with narrow foliage frequently survive an application that kills broad leaved weeds.

Miscellaneous eradication

For eradication of trees, ivy on walls or trees, and many climbers, arsenical pastes containing sodium arsenite are effective. The usual method of application is to drill holes in the wood, fill with the arsenite, and cover or plug with clay.

Sodium chlorate may be applied to gorse as a dry powder, but it is necessary to clean thoroughly any mechanical spreader or duster before and after use. The application of the dry powder is useful in killing other plants also, but an even spread is not so readily obtained as by means of solutions.

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II. SOME FACTORS INFLUENCING THE AGRICULTURAL USE OF CHEMICAL WEEDKILLERS

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I propose to consider first some of the snags in the use of weedkillers by farmers, for with the wealth of chemicals available and the prevalence of weeds, it seems strange that less than 1% of the land under corn in this country was sprayed last year. Although the sellers of most of the chemicals stress, and rightly so, the reasonable cost per acre, of, shall we say, x shillings, this cost can be attained only after a capital expenditure of ys. for a machine. To put things algebraically, y is generally more than 100 times x at the current prices of modern spraying equipment. For this unfortunate state of affairs I am afraid the engineer is to blame, since he has failed to build a machine that is both cheap and durable for the most effective and popular corrosive sprays such as sulphuric acid. Until the price of acid-resistant alloys falls, or that of corn rises, it is difficult to avoid a heavy charge per acre for depreciation. The manufacturers of weed sprays are fully alive to the importance of this problem and have done much to encourage the production of satisfactory machines. They have even proposed the organization of a technical trial with a substantial money prize for the best machine submitted in open competition.

I imagine that some of you are wondering why the farmer is so reluctant to disburse the cost of a machine when most of the published results of randomized plot experiments with sulphuric acid in this country show increased yields of 8–10 cwt./acre

of corn. In terms of money this would amount to an extra return of some 50*s*. an acre in the case of feeding corn and, in the case of the best malting barley, of no less than 150*s*. an acre. These results which concern corn infested with yellow charlock, or white charlock give, indeed, a striking picture of the damage weeds can do. They also illustrate the supreme position held by sulphuric acid as a remedy for white charlock.

I would like to suggest, however, that more information is desirable on the yield increase from more moderate infestations. To illustrate this point I will quote as a comparison the results of some weed-spraying trials carried out in Denmark by Korsmo. It was found, over a period of nine years, that the average increase by acid spraying amounted to only 4 cwt./acre. This result is of great importance to the engineer, since the average gain the farmer is likely to get from spraying determines the price he is prepared to pay for a machine. Would it be possible, by any simple field method, to gauge the intensity of weed infestation in conjunction with crop condition, so as to decide if a crop was worth spraying? I have encountered many farmers with borderline cases. They were not sure whether to spray or leave the crop alone and, almost invariably, the decision went against spraying.

To turn for a moment to an important indirect factor in the development of chemical weed destruction: as I have said, the real depreciation cost of sulphuric acid sprayers is generally excessive. By real depreciation I mean the fall in intrinsic value, as distinct from the conventional depreciation figures used in farm costing. For example, if a tractor is used but little, it will last longer. Experience with sprayers, however, indicates that their working years are numbered immediately they are used for sulphuric acid because of corrosion. The sulphates formed by the acid corrosion of brass and bronze cannot form a protective film on the parent metal as they are very soluble in the spray itself. If, then, a machine is suffering from real depreciation, whether it is used or not, we might naturally ask what other jobs can it do on an ordinary mixed farm? Normally, the answer is that it can do no other useful work. Many attempts have been made to find fresh outlets such as spraying grassland weeds, poultry land infested with coccidiosis, even the spraying of land suspected of carrying the virus of foot and mouth disease has been suggested. All these alternatives have, unfortunately, borne no practical fruit, and it has not been possible to spread the heavy cost of depreciation at all. In a meeting such as this, might I ask you to give this problem of finding alternative uses your consideration? When I said just now that no alternative work has been found for a corn sprayer I was not, strictly speaking, quite correct. As many of you know, Dr C. H. Brown has developed an entirely new use for sulphuric acid in wilting down potato haulm and chickweed to facilitate lifting of the crop. When blight is present this also prevents severe contamination of the tubers from the diseased tops. This spraying, taking place at the end of the summer, would seem an ideal opportunity for increasing the scope of a machine; but spring corn and its weeds are aggravatingly absent from the heavy rich land of the eastern counties where the great bulk of the potato crop is raised. Providence, however, has compensated for this in some small measure by providing an unorthodox use in the potato districts, where acid is also used in the spring to reduce the risk of lodging of heavy cereal crops by destroying some of the flag.

It can be said that, as a whole, the use of machines for sulphuric acid on the rather specialized potato farms to destroy haulm and chickweed is the most profitable

example of chemical spraying, on account of the heavy losses otherwise incurred in a season when blight is prevalent. This is reflected in the better and more costly equipment used there.

I want to turn now to another aspect of chemical weed destruction, namely, the reaction of the farmer. An interesting example was provided some years ago when sulphuric acid spraying was just developing in this country. On a farm in Northamptonshire a certain field, when under spring corn, was frequently almost choked by prolific growths of spurrey. The conventional remedies of liming and spring harrowing were tried without success, and when the harvest came the crop of spurrey seeds was truly amazing. The farmer began to think that perhaps he could grow spurrey more easily than corn, so he despatched a sample of the seed to a firm of chemical manufacturers to find out if it was of any use as a source of oils. A reply came back regretting that it was of no use. Nothing daunted, the farmer sent a sample to a well-known firm who manufactured bird seed, again with a plaintive enquiry. Again came a disappointing reply that the seed was of no value as a constituent even of bird seed. Then, and only then, the farmer rang us up and offered his field for experimental spraying purposes. This viewpoint suggested that spraying is regarded as the very last resource, but it is probable that with the lapse of a few more years a more favourable viewpoint will be developed.

To return for a moment to the idea of our farmer who tried to turn his spurrey infestation to financial advantage: many tons of charlock seeds are obtained in this country every harvest when spring corn is threshed, and the farmer generally burns it. To indulge in a flight of fancy, is it possible that this waste material is worthy of a biochemical investigation as a source of any useful product? I do know of one case where charlock seed is sold for 10*s*./cwt. and used as a constituent of food for game birds, and it is highly prized.

I want now to say a few words about the development of spraying machinery. As many of you know, within the last six years the acreage sprayed annually by sulphuric acid alone, has risen from a few hundred acres up to 30,000. In the last decade we have seen the knapsack sprayer replaced by the cart attachment, the latter in turn by the horse drawn machine with pumps driven from the axle, and to-day the latest development is the power equipped sprayer. This unit has motor driven pumps both to feed the jets and to fill the barrel and, as might be expected, is most plentiful in the potato districts where the financial return from spraying is greatest. This mechanical progress has meant a steadily increasing machine cost rising from £5 to about £100. Working rate at the same time has risen from about 1000–12000 sq. yd./hr., while manual labour has conversely decreased, falling in cost from about 4*s*. to 9*d*. an acre.

At Oxford, investigations on acid-spraying machinery have proceeded along three main lines. The first has been to improve the methods of handling acid and water in the field. One object of this was to reduce the idle time of the sprayer on the headland during filling, and has, in fact, by suitable proportioning of pump sizes, given an increase in working rate of 28% with a concurrent rise in capital outlay of 10%, which may be regarded as satisfactory.

The second benefit from improved handling methods, is to reduce risk of splashing and burning, and generally make spraying a reasonably clean job that farmers will not dislike. If, as seems possible, some producers of sulphuric acid find that contract

spraying is not remunerative, it is important that the farmer should be willing to tackle the job himself.

The second line of attack has been on the corrosion and depreciation problem. The alloy 18 : 8 nickel chrome steel has proved a resistant material under all field conditions. Unfortunately, despite its present reasonable price, no commercial machines yet embody it, as the great toughness of this alloy demands special tools and more time to machine it which, together, make it too expensive. The obvious solution to corrosion, that of cleanliness, has not proved successful in practice, partly because of the difficulty of cleaning machines properly without dismantling them.

The third line of attack has been on the water consumption. It is surprising to reflect that when a farmer sprays 50 acres of corn no less than 16-18 tons of water have to be pumped and carted. It has been observed that in the wheel tracks of a machine the weed kill is almost always 100 %. This led to an experimental machine with rollers but, unfortunately, it was found that a reasonably light roller which the horses could pull did not reproduce the effect of the sprayer wheels—presumably because the ground pressure on the weeds was too low. This line is capable of development were a tractor available to pull the machine. Concurrently, the relationship between pump pressure, nozzle contour, and the size of the spray droplets, was examined. Smaller droplets may reduce the volume of spray required per acre, but will increase the draught of the sprayer by demanding an increase in the pressure drop through the jets.

To sum up, the wet sprayer still lags behind in comparison with other modern farm machinery. It is too small to take advantage of tractor power and has, consequently, a low working rate. That these technical snags can be overcome there is little doubt but, unfortunately, the bogey of capital cost, coupled with the fact that weeds are awkward irregular things, will, I think, continue to restrict further mechanical development of the sprayer that is used solely against them.

To turn now to the sphere of dry spraying or dusting; here the problem of the machine is much less acute. It may cost £35, but it has uses other than weed destruction and, consequently, its depreciation charge on any particular job is much reduced. Frequently, an existing farm distributor can be used and fresh capital outlay avoided, a factor whose value can hardly be over-estimated in farming.

We are all aware of the advantages of dry weed destruction. There are no difficulties in regard to a water supply and, concurrently, there is no serious corrosion problem. On the other hand there is not the clean and complete extinction of the weed which the wet method gives.

In part, this incomplete weed destruction is a mechanical problem, since better results are always obtained when the powder is applied evenly over the whole of the plant surfaces. This ideal is never obtained with an ordinary farm distributor, which either dresses the crop with narrow and regular bands of powder or, what is worse, with haphazard little teaspoonfuls here and there, giving one weed too much and another no powder at all. Distribution which is perfectly uniform when expressed as weight of powder/sq. yd. can be hopelessly irregular when expressed as weight/sq. in.

Frequently, however, by fitting to a machine little paddles that break up the falling streams of powder, much more uniform results can be obtained, at an additional expenditure of about £5.

The working rate of a typical distributor is about the same as an axle driven sprayer, but the distributor can only work effectively for 3–5 hr. a day while the dew is on the weeds.

The cost of applying dry powders such as cyanamide in this manner is about 1*s*. 6*d*. per acre, while to apply acid costs about 4*s*. 6*d*. and possibly more. The overall cost of the wet and dry methods are roughly the same, however, as the acid costs less than the cyanamide. The cyanamide, of course, gives the crop about 100 lb. of lime and 30 lb. of nitrogen to the acre, but the latter constituent is, unfortunately, not often wanted by the grower of malting barley.

Within the last five years equipment for dry spraying has advanced considerably and, by dispersing powders in air before applying them to the crop, much better weed destruction is attained. In addition, machines are available that can work at the rate of about 50,000 sq. yd./hr., far surpassing any wet sprayer in output.

III. THE RELATIVE TOXICITY OF CHEMICAL WEEDKILLERS

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During the last 30 years a large number of substances have been tested for their effectiveness in destroying unwanted plants. To-day the compounds which are most widely employed in agriculture and horticulture can, in regard to the nature of their action, be divided into three categories. There are, firstly, compounds such as sulphuric acid or sodium hydroxide which are only successful when applied in comparatively strong concentrations, since their destructive power is dependent on the solution being either strongly acid or alkaline. The second group, which is by far the largest, contains compounds which are effective in dilute concentrations, since they or their ions accumulate in toxic amounts within the plant. In the third group, the compounds are neither directly toxic in dilute concentration nor are the solutions acid or alkaline. Their action is dependent on a high solubility in water and, in consequence, plasmolysis of the plant tissues. I do not propose to discuss in any detail the comparative effectiveness of substances either in the first or last group. Recent research has indicated, at least where the acid substances are concerned, that they are equally efficient as long as the hydrogen ion concentration is of the same order. But, in the second group, our knowledge of relative toxicity is far less certain for, in the past, few experiments have been carried out in which several compounds have been tested simultaneously under a wide range of conditions.

In attempting to assess the merits of various compounds several difficulties confront the experimenter. It has been shown repeatedly that species vary enormously in their susceptibility to any one compound. For example, we have found at Jealott's Hill that, even within a single genus, there are wide divergencies. A dressing of sodium chlorate at the rate of 60 lb./acre will almost completely eradicate *Plantago coronopus* and *P. lanceolata* from pastureland. Yet the same dressing will only partially control *P. major*, while *P. media* is completely resistant. Again, in some species the season of the year must be taken into account. For the control of *Cnicus*

arvensis an autumn application of sodium chlorate is more effective than a spring one, but in the case of *Hypochaeris radicata* the time of application is immaterial. Moreover, weather conditions must also be borne in mind. Copper sulphate is ineffective for the eradication of *Brassica arvensis* in showery weather, while sodium arsenite sprays give the best results during times of drought.

In order, therefore, to obtain a true comparison between several substances experiments were carried out over a range of both season and weather conditions. The chief plant investigated was *Senecio Jacobaea*, since there was evidence that it was susceptible to a member of the more well-known weedkillers. Among the substances tested were aluminium sulphate, aluminium nitrate, ammonium thiocyanate, copper nitrate, copper sulphate, sodium arsenite, sodium bichromate, sodium bisulphite and sodium chlorate. The compounds were either applied in the autumn in powder form (sodium arsenite in solution), or as sprays (100 gal./acre) at different times during the growing season. The first experiments demonstrated that the aluminium salts, the copper salts, sodium bisulphite and ammonium thiocyanate were all entirely ineffective. In the later experiments these, with the exception of ammonium thiocyanate, were omitted. These later experiments, however, confirmed the earlier results for ammonium thiocyanate since even when the rate of application was as high as 300 lb./acre only 20% of the plants were killed. Of the remaining three compounds, sodium chlorate, sodium bichromate and sodium arsenite, the first gave by far the best results. Irrespective of either the season or the weather conditions, sodium chlorate at a rate of 50–60 lb./acre gave a control of 88–100%. Sodium bichromate was only effective at much higher concentrations; complete suppression was not obtained until the rate was raised to 300 lb./acre. The results for sodium arsenite were very variable; applied in amounts equivalent to 13–15 lb. of arsenic trioxide per acre the control ranged from 0 to 72%. The efficiency was greatest during dry weather when the plants showed signs of wilting. The effectiveness of sodium arsenite and other compounds was not increased by the addition of wetters to the spray.

While these experiments have established for *S. Jacobaea* that sodium chlorate is the most toxic of the weedkillers tested, it does not necessarily follow that this result holds for other species. Only further experiments along the lines of this investigation can settle whether the chlorates are the most effective weedkillers for other plants.

IV. THE CONTROL OF WEEDS IN LAWNS AND FINE TURF

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In the space allotted in this symposium it is only possible to cover the field in general terms but it cannot be covered adequately by describing chemical methods of control only, because good management demands various supplementary operations some of which are mechanical. It should be realized also that turf weeds are not solely non-gramineous but that a number of pernicious weed pests are grasses. Furthermore the establishment of new turf in a weed free condition, the prevention of weed invasion, the use of grass species or strains which are aggressive enough to resist weed invasion,

are all important aspects of this problem. Weed invasion and weed eradication are closely related but there is no single all embracing method which can be advised to suit all conditions. Perhaps the problem may best be understood by first of all giving a brief summary of the requirements of a good turf.

Requirements

A good turf must be dense, uniform in texture and true because trueness means even mowing and thus greater uniformity. If the turf consists of more than one species then even blending is important. The surface should be carpet-like and there should be some resiliency to the foot. To be successful the sward should consist of dwarf, fine-bladed grasses, capable of making bottom growth and it should consist predominantly of species of bent (*Agrostis*) and fescue (*Festuca*). To produce this state of perfection demands intensive management. The commonness of bad turf areas is due to neglect and a failure to appreciate the amount of work necessary to produce the high degree of excellence desired by many but attained by few.

Mowing and weeds

Of all the operations of maintenance, mowing is perhaps the most abused. Mowing determines very largely the species of grass in a turf and it is because of the mowing that the weed problem often becomes so acute. In the finest lawns mowing three times per week is not excessive and many modern golf courses arrange for mowing to be carried out seven days a week in the height of the season. The constant defoliation removes competition and enables weeds to become established, many of which are able not only to persist but rapidly increase under conditions of keen mowing.

Four classes of weeds may be considered:

(1) The mat weeds consisting of such species as pearlwort, mouse-ear chickweed, yarrow, selfheal, creeping buttercup and bedstraw, all of which spread by runners and form a dense mat. The mosses are perhaps best included in this group.

(2) The flat or rosette weeds containing for example four species of plaintain, daisy, cat's-ear, and the erodiums, some of which spread by budding out daughter plants.

(3) The clovers, principally creeping clover and yellow suckling clover (the latter spreading by seeding).

(4) The annuals, parsley piert, shepherd's purse, chickweed, groundsel, peculiar to new lawns, certain grasses such as Yorkshire fog, annual meadow-grass, perennial rye-grass, and other coarse species.

Few annuals persist in regularly mown turf, the exceptions being such plants as annual meadow grass and parsley piert which flower and set seed below the level of the mower blade.

Although mowing may be a primary factor in determining the species of plants able to persist in fine turf, the species of grass present and even the strain have an influence in determining the incidence. A dense matted species inhibits invasion to a large extent; furthermore soil factors are partly responsible. The effect of species is shown by the following figures for plots of the same age and treated alike:

Chewing's fescue (*F. fallax*) plot: 63% grass, 19% weeds.
Browntop (*A. tenuis*) plot: 59% grass, 9½% weeds.

New lawns

Faulty establishment of new lawns is often the cause of future trouble and perhaps the commonest error is insufficient fallowing to secure a clean seed bed. Impure seed is also another source of trouble, and failure to sow uniformly may assist weed entry. The seed rate when sowing a new lawn may be from 3 to 6 cwt./acre, and all efforts must be directed to secure a rapid and uniform establishment of the grass by thorough cultivation and pre-treatment of the soil so as to ensure a density sufficient to keep down any surviving annual weeds.

In view of the high seed rate the use of pure seed only is exceedingly important; for instance a sample of Chewing's fescue seed containing 2·5% of grass and non-grass impurities when sown resulted in the sward being only 75% pure with 22% of grass weeds and 3% non-grass weeds. A similar sowing using browntop seed containing 1·6% of grass impurities resulted in a turf only 63% pure with 27·5% of Yorkshire fog and other grass weeds, and 9·5% bare ground.

The importance of clean seed may be realized by expressing the matter in another way. Thus, if browntop containing 0·1% of mouse-ear chickweed is being sown at 2 oz./sq. yd. (about 6 cwt./acre), there will be approximately 550 seeds sown on each square yard. Again if 2 oz./sq. yd. of Chewing's fescue containing 0·2% perennial rye-grass is sown to the square yard, then 57 seeds would also be sown. The presence of even one or two plants of perennial rye-grass per square yard in a fine turf is sufficient to cause considerable expense in eradication on anything but very small areas. It is not entirely the weight of weed seed present but their number and nature as well as their potential danger which decides whether or not the grass seed is suitable for use.

Many new lawns are established with rye-grass mixtures and weed invasion is usually rapid since rye-grass rarely forms a dense turf. Where a percentage of browntop is included bottom growth is formed which then appears to accelerate the gradual decline of the rye-grass under conditions of keen mowing. Thus a plot sown with indigenous perennial rye-grass, smooth stalked meadow-grass, and crested dogstail, consisted after 4 years of 45% rye-grass, 21% other grasses, 26% weeds, whilst the adjacent plot sown with the same seeds and 50% of browntop, contained at the end of the same period only 5% rye-grass, 5% other grasses, 71% bent, and 15% weeds. In new sown turf where the soil has been poorly cleaned the occurrence of annual weeds like chickweed, shepherd's purse and groundsel is not uncommon. Provided these weeds are not too extensive, they gradually die out as a result of regular mowing though in some cases with autumn sowings chickweed may seriously assert itself in the interval before mowing is possible the following spring.

Established lawns

Having established a turf of pure seed it is necessary that treatment should be directed to maintaining it in weed free condition. Much attention has been devoted to this problem in the United States and the work has very largely been repeated at St Ives with substantially the same results. For example, by regularly fertilizing pure sowings of Chewing's fescue and browntop, with ammonium sulphate, chloride, or phosphate, or with sulphate of ammonia plus superphosphate with or without potash, it has been possible to maintain the turf in weed free condition over a period of

8 years. The addition of sulphate of iron assists the process. On the other hand the effects of the continuous use of nitrate of soda, nitro-chalk and nitrate of lime, and the above ammonium fertilizers with lime has led to the invasion of other grass species and weeds. Where Yorkshire fog is present however it is able to persist under mown conditions even when ammonium compounds are used.

Examples (Table I) from the botanical analyses taken in 1937, on plots started in 1929 may be of interest.

Table I

Plot no.	Species sown	Treatment	Year started	% fescue	% other grass	% weed
1	Fine-leaved fescue	S/A S/I	1929	99·5	0·0	0·5
2	,,	S/A S/I lime	1929	3·0	60·5	36·5
7	,,	P.K. S/A	1929	91·0	9·0	0·0
8	,,	P.K. S/A + lime	1929	0·0	18·5	81·5
1	Chewing's fescue	No fertilizer	1929	52·5	6·0	41·5*
2	,,	S/A	1929	89·5	10·5†	0·0

S/A = sulphate of ammonia.
S/I = sulphate of iron (calcined).
* = containing 37% moss.
† = *Holcus* sp. and *Agrostis* sp.

Effects of mowing

As already mentioned, the general effect of mowing is to remove the competition of the grass and to permit weed invasion. The return or removal of the cuttings on each occasion also has an effect on the nature of the sward. Thus, on an area to which the cuttings are always returned the amount of annual meadow-grass tends to increase and stands at 22%, as against the adjacent plot from which the cuttings are always removed where the percentage of this grass is only 2%. No doubt this difference is largely due to the return of seeds from the panicles of the annual meadow-grass but the softer and moister soil where the cuttings are returned may also favour this volunteer species.

Experiments are in progress at St Ives to determine the effect of height of cut (three levels) on weed invasion and upon the general condition of the turf. This work still continues, but it has been found that closer cuts give a denser turf and, indeed, with the longer cuts, the amount of weed tends to increase within the limits of the experiment.

Table II shows the comparisons of weed percentages (1937 figures) on certain of the plots started in 1931.

Table II

Treatment	Height of cut inches	% weeds	% moss	% grass	% bare ground
N/C + compost	$\frac{1}{8}$	2·5	19·0	78·0	0·5
N/C + compost	$\frac{3}{8}$	23·0	0·0	70·5	6·5
S/A + compost	$\frac{1}{8}$	0·0	1·5	98·0	0·5
S/A + compost	$\frac{3}{8}$	0·0	0·0	98·0	2·0
Compost only	$\frac{1}{8}$	1·5	34·0	64·5	0·0
Compost only	$\frac{3}{8}$	8·5	11·0	80·0	0·5

It will be noted that on the shorter mown plots there is a higher percentage of moss even though the number of shoots per unit area has been found to be greater and, possibly, this may be correlated with the degree of shading.

Experiments on weed eradication

A brief consideration may now be given to weed eradication as distinct from weed control or inhibition. In 1931 an area of weedy turf was subdivided for various manurial treatments and a series of nitrogenous dressings applied, both with and without sulphate of iron since previous work had shown that the effect of adding sulphate of iron to nitrogenous manures was to accelerate the reduction of weeds. It was found that all forms of nitrogen lead to a reduction in the weed population but the largest reduction, which in most cases was complete, took place with sulphate of ammonia and ammonium phosphate and least with dried blood, urea, and cyanamide, whilst nitro-chalk and nitrate of soda came in between. The addition of sulphate of iron even to materials like nitro-chalk and nitrate of soda leads to increased weed reduction, and the use of sulphate of iron alone in amounts considerably greater than those used with fertilizers results in marked reduction of such species as daisy, ribwort plantain and selfheal. A full account of this work is in preparation.

Other experiments have been specifically concerned with the control of clover in fine turf, and Table III shows the effects of certain regular treatments started in Autumn 1931.

Table III

Treatment	% weeds	% clover	% moss	% grass
Control	2	22·0	32·0	44
S/A compost	0	T.	3·0	97
N/S compost	6	0·5	32·5	61
S/I only	0	34·0	8·0	58

N/S = nitrate of soda.
T. = trace.

The figures shown in Table III were obtained in 1937 but are substantially the same as those secured in 1933. It will be noted again that the sulphate of ammonia has a more striking effect than nitrate of soda, whilst on the sulphate of iron plot there is more clover than on the control. This may be due to the fact that regular use of sulphate of iron leads to a thin open turf and conditions more suitable for the spread of this weed.

Practical methods of weed eradication

Practical methods of weed eradication nearly always entail the use of sulphate of ammonia or ammonium phosphate with or without sulphate of iron, and if there is a foundation of fine grass, then the use of these materials will, after a number of dressings, result in rapid elimination.

The common formula used on lawns consists of:

3 parts by weight sulphate of ammonia.
1 part by weight sulphate of iron (calcined or fine crystals).
20 parts by weight carrier (such as potting soil or compost).

This mixture is applied six times per annum at 4 oz./sq. yd. This mixture is particularly effective against daisy, selfheal, pearlwort, and chickweed but, for dealing with cat's-ear, dandelion, and plaintain, a more concentrated mixture is applied. This consists of:

3 parts by weight sulphate of ammonia.
2 parts by weight sulphate of iron.
5 parts by weight carrier such as dry sand.

This mixture is used broadcast at 3–4 oz./sq. yd. or as a spot treatment. Ammonium compounds give a fair degree of selectivity, and if careful application is made damage to the grass is, usually, only temporary. Weed control by this process may be attributed to a combination of factors, for example nitrogen effect on the grass, increased soil acidity, direct plasmolysis and ammonium toxicity.

For weeds like moss and pearlwort, larger quantities of sulphate of iron are usually employed and a mixture of equal parts of dried blood and sulphate of iron is commonly used for controlling pearlwort. For moss control careful raking, followed by improvement of the fertility of the soil is adopted since moss is usually a sign of neglect and poverty rather than of dampness. Forking plus light liming where tests indicate, are often supplementary but at times attention to drainage may be necessary. Extensive experiments have been carried out with permanganate of potash at St Ives for moss control. Reduction is mainly temporary unless very heavy rates are used, the cost then being 5 or 6 times more than for sulphate of iron.

For large scale work on playing fields, large lawns, and gold fairways, sulphate of ammonia and sulphate of iron are commonly used without carrier; a mixture of 3 or 4 to 1 at 1–2 oz./sq. yd. applied with special types of distributors is very efficient. Other lines of work which are being investigated, following work in New Zealand, are spraying with arsenic acid at strengths from 1 in 60 to 1 in 120, according to the species of weed concerned.

For controlling annual meadow grass, dilute arsenic acid spraying appears to have possibilities though it has the disadvantage of causing a complete browning of the turf. Where weeds are extensive, renovation after this method is of course necessary and, indeed, on any area where the weeds have been excessive.

Experiments with chlorates and thiocyanates have been carried out but have not been sufficiently striking to warrant extensive work. More recently solid cones containing sodium chlorate have been put on the market and these are inserted into the crowns of the weeds. For rosette weeds they are very efficient and damage to the surrounding turf is kept to a minimum. Other large scale methods of killing rosette weeds on golf courses entail the injection of dilute arsenic acid into the crowns of the weeds and, by applying a few drops of the acid, through an oil-can, to the centre of the rosette.

As already mentioned, the control of weeds in fine turf does not involve solely the use of chemicals, since mechanical operations are frequently necessary either alone or supplementary to chemical treatment. Thus, for moss, occasional raking is desirable and, for the control of Yorkshire fog since no selective chemical method of eradication is yet known, the present method is to carry out systematic slashing followed by renovation with fine seeds and an improvement of the fertility. The raking of all mat weeds is desirable and particularly is this so in the case of creeping clover, the runners of which must always be raken up and removed with the mower. For weeds like chick-

weed constant use of a drag brush on the turf prior to mowing is an advantage, and to control annual meadow-grass drag brushing prior to mowing is beneficial. The same treatment has been adopted successfully for the control of rough-stalked meadow-grass. Mechanical operations are also useful in controlling yarrow which may be closely shaved by a scythe or special hoe, made for the purpose, and also by slashing the stems and underground parts with special slitting tools. Close "shaving" of clover in hot weather followed by light applications of sulphate of ammonia is effective in reducing this weed on most soils.

V. CHLORATE WEEDKILLERS

By O. Owen, M.Sc., Ph.D., A.I.C.

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Whilst previous contributors have dealt with the efficacy of various chemicals as weedkillers this contribution deals briefly with injury which has resulted from the careless use of a very popular weedkiller, namely sodium chlorate. Except for the fire risk involved in its use sodium chlorate appears to have all the properties of the ideal weedkiller, and this has led to its use on an increasing scale in nursery and market garden work. Its high toxicity and the fact that it is not specific in its action have, on many occasions, caused nurserymen serious losses.

The first case which was brought to our notice concerned a tomato nursery in which a large number of plants were affected. The foliage showed a definite mottle, older leaves were desiccated and brown lesions were apparent on the stems: the symptoms generally were those associated with what was then known as "stripe" disease. The effects were confined to two well-defined areas in two houses. The application of fertilizers and repeated planting had been of no avail. Eventually the injury was found to be due to chlorate in the soil, the concentrations varying from 0·02 to 0·03% calculated as sodium chlorate. Steam sterilization caused a definite improvement in the condition but the beneficial effect was not permanent, and it was not until two years later that the injurious effects had completely disappeared: this despite very heavy floodings.

Since then cases dealt with have been many and varied; at some time or another practically all the plants raised in nursery work have suffered.

A common instance is that in which paths outside tomato houses have been treated with chlorate for the eradication of weeds. Rows of plants adjacent to the boundary walls often show effects of chlorate poisoning. An interesting case in this connexion is one where a group of plants 12 ft. away from a wall were showing mild but definite symptoms of injury. The paths outside the house had been treated six weeks previously. The plants showed ill-effects within a few days of the first heavy rainfall since the chlorate had been applied.

Another source of danger is that which attends the growing practice of treating "standing-out" ground with chlorate. This may cause injury in two ways. In the first the pots may become contaminated without actually affecting the plants in them, e.g. heaths which are comparatively resistant to chlorate. When, however, the pots are brought into houses enough chlorate may be washed off the pots into the soil to injure a succeeding crop, such as tomatoes, planted in the soil. Then, of course the pots may themselves be used for other plants of a higher susceptibility with con-

sequent damage. There is, also, the great risk that plants such as chrysanthemums may be affected. We had one case of extensive injury on a nursery where chlorate had been used for some years with satisfactory results. The damage reported was found to be due to the fact that a workman had omitted to wash in the weedkiller which had been applied in the dry state. Another case of chrysanthemum injury concerned a grower who had treated the "standing-out" ground in the spring of 1936. Later in the year 4000 chrysanthemums were stood out and proved a complete failure. In the late autumn the grass on the ground was quite green again and in 1937 it was cut two or three times but again the crop was a total loss. The pots had taken up and retained sufficient chlorate to injure the plants.

It is interesting to note that injury to chrysanthemums is often confined to the tops where the leaves become a peculiar grey-green colour, with considerable distortion and some shrinking, while the lower leaves exhibit little or no abnormality.

One other case may be of interest. Many crops in a kitchen garden were thought to be suffering from a virus disease, but examination of the soil showed the presence of a chlorate despite repeated assurances that no chlorates had been used near the garden in question, nor had any watering cans been used for applying chlorate. Eventually it was found that water was drawn from a pond in another part of the garden and this water had become contaminated by drainage from paths which had been treated with chlorate.

There is a definite variation in the susceptibility of different cultivated plants to chlorates. Of those met with here, heaths definitely show the least susceptibility. At the other end of the scale tomato plants, dahlias and winter cherries are among the most susceptible. During sunny weather under glass a young soft, tomato plant will show signs of injury well within 24 hr., with concentrations of the order of one part of sodium chlorate per ten thousand of soil.

In addition to the plants already mentioned the following plants have, among others, been found to be suffering from chlorate injury: garden nasturtiums, wall flowers, cinerareas, asters, cucumbers, lettuce, antirrhinums, primulas, geraniums, sweet peas.

Generally, where the poisoning is mild the plants show a distinct mottle which may be (and actually has been) mistaken for a virus effect. Where injury is more severe, distortion of the foliage and desiccation occur. More detailed information concerning effects and concentrations producing them will be found in the *J. Minist. Agric.*, 1937, **44**, No. 6, p. 866.

REVIEWS

Introduction to Plant Pathology. By F. D. HEALD. Pp. xi + 579. London: McGraw-Hill Publishing Co., Ltd. 1937. 24*s.* 0*d.*

In 1933 Prof. Heald published a second edition of his *Manual of Plant Diseases*, a standard American treatise running to 953 pages, costing 45*s.*, and intended for serious students. A shorter, cheaper, and more elementary introduction to the subject has been needed and Prof. Heald has filled the gap. The new volume resembles the *Manual* in many ways, but it is not a mere abridgement; the material has been rewritten, new matter is included, and there is a different order of presentation.

The book is divided into six sections, the first being introductory. Ch. I deals briefly with the nature of plant disease and the development of the subject, mostly in America. Ch. II is an excellent summary of the symptoms of disease in plants, although one would have liked to see this subject treated more on a developmental basis. Ch. III discusses the relation of fungi and bacteria generally to human affairs. This is an interesting innovation and contains much useful material, but some of it seems a little out of place in a text-book specifically of plant pathology. Ch. IV deals with the nature and extent of plant disease losses. Ch. V concerns the dissemination of plant-diseases.

Section II, devoted to the parasitic diseases, comprises ten chapters. Ch. VI introduces the student to the vegetative and reproductive structures of fungi, and the following nine chapters deal respectively with diseases due to Phycomycetes, Ascomycetes, Ustilaginales, Uredinales, Hymenomycetes, Fungi imperfecti, bacteria, parasitic seed plants, and nematodes. The general method of treatment in each chapter is to outline the structure and classification of the particular group, describe selected diseases in some detail, and then tabulate the diseases and parasites. Thus, Ch. VIII contains two introductory pages on the Ascomycetes; detailed considerations of peach leaf curl, brown rot, anthracnose of currant, powdery mildew of apple, ergot, black knot, and apple scab; and a table of 121 important diseases due to Ascomycetes. A more or less standard mode of presentation is adopted for each disease: history and distribution, symptoms and effects, crop losses, etiology, pathological anatomy, climatic relations and predisposing factors, host relations and varietal resistance, prevention and control, references. The selection and ordering of the material in these chapters is excellent.

Section III comprises two chapters on viruses and virus diseases. The first deals generally with the types of viruses, methods of transmission, and the nature of viruses; and the second contains descriptions of specific virus diseases, and a list of 241 diseases of 141 host plants. Section IV is devoted to the non-parasitic diseases, which are discussed in four excellent chapters as follows: diseases due to unfavourable soil conditions, and deficiencies or excesses of food materials; diseases due to improper air, temperature, and light relations; diseases caused by manufacturing or industrial processes; and diseases due to control practices. Section V concerns the prevention and control of plant disease and, following an introductory chapter, three chapters are devoted respectively to disinfesting practices, sanitary and cultural practices, and seed selection and the selection and breeding of disease-resistant varieties. Section VI deals briefly with phytopathological technique and contains three chapters devoted, respectively, to methods of studying parasitic, virus, and non-parasitic diseases. The book opens with a synoptic contents, and closes with an index which omits reference to diseases and parasites merely listed at the ends of chapters.

The selection and arrangement of the material and the balance of the sections seem to me admirable, and although the volume is arranged on a mycological and not a host basis it is a real introduction to plant pathology and not merely a text-book of semi-applied mycology. The author's style is clear and simple, but he is not entirely

consistent in his terminology and, occasionally, e.g. at the top of p. 351, his pen runs away with him. The book contains many Americanized spellings, and misprints have been noted on pp. 19, 39, 56, 114, 160, 163, 217, 249, 270, 301, 497, and 566. Students may find the bibliographies annoying, since, for investigations published before 1932, reference is merely to the larger *Manual*. The 200 illustrations are of good quality and well chosen, but there seems no reason for the waste of space on pp. 235–7. As the book is written for American students, American diseases, naturally, are emphasized, and certain diseases which would find an important place in an equivalent English course are either omitted or merely noted; e.g. silver leaf of plum, wart disease and powdery scab of potato, yellow rust and whiteheads of wheat, leaf stripe of barley, clover rot, chocolate spot of bean, American gooseberry mildew, spotted wilt of tomato, reversion of black currant, etc. Other matters of interest are the use of small letters for all specific names, the adoption of the genus *Monilinia* for the monilioid pseudosclerotial *Sclerotinias*, and the inclusion of *Plasmodiophora* and *Spongospora* in the Chytridiales, and *Actinomyces* in the Hyphomycetales.

Prof. Heald is to be congratulated on having written perhaps the best *Introduction to Plant Pathology* yet published.

WILLIAM B. BRIERLEY.

Der Schwarzrost: seine Geschichte, seine Biologie, und seine Bekämpfung in Verbindung mit der Berberitzenfrage. By E. LEHMANN, H. KUMMER and H. DANNENMANN. Pp. xxiv+584, 1 colour plate and 87 text-figures. Munchen/Berlin: J. F. Lehmanns Verlag, 1937. Rmk. 21 (cloth-bound); Rmk. 19.50 (paper covers).

There are numerous books on general plant pathology, on the diseases caused by one or another group of pathogenic agencies, on the diseases of particular crop plants, or on particular groups or genera of parasites, but the present book is something new in plant pathological literature in that it is a treatise of very respectable size devoted entirely to one disease. The only other publications of somewhat similar nature I am acquainted with are McAlpine's work on bitter pit of apple, and Falck's work on dry rot of timber, but these are more progress reports than complete and independent treatises. The publication of *Der Schwarzrost* sets a new standard in plant pathology and, in a way, marks the commencement of a new period; a period of specialization on individual diseases rather than on individual crop plants, or individual genera or groups of parasites; one hopes that the present work may be but the first of a series of such monographs. So commanding a volume could hardly be written on any other single disease, since black rust is probably the most important plant disease in the world, but potato blight might run it very closely and shorter monographs of similar type on, e.g. club root of crucifers, wart disease of potatoes, downy mildew of the vine, apple scab, ergot, bunt of wheat, silver leaf, crown gall, fire blight, low or high temperature injury, etc., are immediately feasible and would be of great value.

Black rust of wheat has received an enormous amount of attention, as may be gauged from the fact that the Bibliography in the present volume contains some 2000 references in all sorts of languages and, even so, it is not complete. The reviewing of this mass of work, which is very heterogeneous and of uneven quality, and the ordering and considering of the data must have been a Herculean task. The authors have done their work admirably but their very conscientiousness has led them into perhaps unnecessary length and, here and there, a little more severe and critical handling of some of the work might have been desirable. Every facet of the subject is considered in detail but, in reading the book, one never gets a sense of undue padding or of false values.

Ch. I is a mere introductory note. Ch. II (37 pages), is an interesting historical survey of the subject, commencing with ancient biblical times and ending with biotypes: it is also, to a certain extent, a very brief summary of the book itself.

Ch. III (27 pages), concerns the world distribution of the barberry, and Ch. IV (47 pages), its indictment in various countries. Some of the matter in these two chapters seems a little unnecessary, but the detailed consideration of the barberry may be justified by the fact that, in central and western European countries far more than in many other wheat regions of the world, the barberry retains its primary importance as an obligatory alternate host of the parasite in relation to outbreaks of black rust. Ch. V (228 pages), is a very detailed consideration of the biology and physiology of parasitism of *Puccinia graminis* in both graminaceous hosts and the barberry. Every phase of the problem is dealt with in a remarkably efficient way, and these pages contain the most thorough discussion by far of any particular aspect of disease I am acquainted with in plant pathological literature. They are, as it were, a complete "Fischer & Gäumann" of black rust. In Ch. VI (27 pages), the epidemiology of black rust is considered and, although some first class work has been done on this problem, especially in America, the inadequacy of our knowledge is very evident. It is a curious fact that, although plant diseases are especially suitable material for epidemiological study, yet, epidemiology remains one of the most neglected aspects of plant pathology. Ch. VII (40 pages), gives an account of the world distribution of black rust, and of the available but inadequate data on the damage it causes. Ch. VIII (106 pages), is an excellent account, with especial reference to barberry eradication, of the practical measures which have been adopted in various countries to control black rust. The book closes with a useful Bibliography (57 pages) and a somewhat slight Index.

So densely packed and encyclopaedic a treatise might easily have lost much of its value by inefficient arrangement of material but, fortunately, the work is well organized and a detailed Contents, running to 14 pages, makes it easy to find any particular information one needs. This book is a work of quite outstanding merit, conceived and written in the older and greater German scientific tradition. It sets a standard for all monographs of similar nature on plant diseases, and it will long remain the primary source of knowledge and inspiration for workers on black rust.

WILLIAM B. BRIERLEY.

The Structure and Development of the Fungi. By H. C. I. GWYNNE-VAUGHAN and B. BARNES. 2nd edition. Pp. xvi+449, with a Frontispiece and 309 text-figures. Cambridge: University Press. 1937. 18*s*.

The first edition of this well-known book, published in 1927, received notice in the *Annals* 1928, **15**, 150. The new book shows slight rearrangements but follows closely the lines laid down in the first edition: two short introductory chapters on general fungal morphology and classification, and on fungal physiology and forms resembling fungi; three long chapters containing a detailed consideration of the Phycomycetes, Ascomycetes and Basidiomycetes; two pages on the Fungi imperfecti; a short chapter on mycological technique; a bibliography and an index.

During the period between the editions the whole front of mycology has advanced: the details of the life histories of numerous fungi have been elucidated, heterothallism has been discovered in the rusts and found to be of widespread occurrence in the fungi generally, and there has been greater realization of the importance of flagellation as a guide to interrelationships in the Phycomycetes. This progress is reflected in almost every page of the book, the new knowledge having been digested and assimilated skilfully in the present text. As in the first edition, the authors are severely logical in their treatment and conservative in their outlook; the book is a model of condensation. The number of illustrations has increased from 285 to 309, the bibliography from 29 to 42 pages, and the index from 18 to 25 pages.

The new edition will enhance the high reputation of its authors and receive a warm welcome from all academic students of the fungi.

WILLIAM B. BRIERLEY.

A First List of Cyprus Fungi. By R. M. NATTRASS. Pp. xvi+87. Nicosia: The Government of Cyprus. 1937. 2*s*.

The introduction contains a brief account of the topography, meteorology and vegetation of Cyprus, with general notes on the relative incidence and distribution of the fungi. The list comprises 351 named organisms classified as follows: Bacteria, 5; Archimycetes, 1; Phycomycetes, 20; Ascomycetes, 37; Ustilaginales, 19; Uredinales, 91; Hymenomycetes, 35; Hyphomycetes, 87; Melanconiales, 15; Sphaeropsidales, 41. There are six new species with Latin diagnoses, two new combinations, and one new variety. The fungi are mostly pathogenic, and a portion of each collection, on which identification is based, has been deposited in the Imperial Mycological Institute, Kew. The fungi are listed alphabetically within their respective groups, with notes on spore measurements, locality, pathogenicity and host range. There are a host index of 236 plants with their fungal parasites; a short bibliography; 15 plates with drawings of the fructifications and spores of 49 fungi; and a topographical map of Cyprus showing crop regions. The genus *Acrostalagmus* is retained; *Hormodendrum cladosporioides* is listed as a fungus distinct from *Cladosporium herbarum*; small letters are used for all specific names; and there are occasional wrong spellings, e.g. "*Beauvaria*" for *Beauveria*.

WILLIAM. B. BRIERLEY.

(1) *An Introduction to Economic Botany.* By J. GILLESPIE. Pp. 96. 1937. 1*s*. 6*d*.

(2) *An Interpretation of Biology.* By H. COLLIER. Pp. 96. 1938. 1*s*. 6*d*. London: Victor Gollancz, Ltd.

These are volumes III and VII, of "The New People's Library". The publisher states: "The aim has been that each book (*a*) should be authoritative, (*b*) should be simply written, (*c*) should assume no previous knowledge on the part of the reader."

Mr Gillespie's book is not concerned with the subjects usually implied by the term "economic botany": it is an introduction to general botany and to some of its applications in agricultural and horticultural practice. The subject matter is not too well arranged and contains occasional inaccuracies and ambiguities.

Mr Collier's book is a rather polemic introduction to human biology.

Both books are written in a colloquial style and illustrated by unnecessarily crude diagrams.

WILLIAM B. BRIERLEY.

Farm and Garden Seeds. By S. P. MERCER. Pp. 205. 1938. 10*s*. 6*d*.

In view of the importance to the farmer and grower of seed testing and weed seed impurities, and of the fact that seeds legislation has been in existence in this country since 1869, it is surprising that so little has been written specifically upon this subject: "Parkinson & Smith", "Remington", and odd chapters and pages in Percival's *Agricultural Botany*, Fream's *Elements*, Hunter's *Encyclopaedia*, and in other general works. On the continent of Europe the subject has received much more the attention it deserves.

The author of the present book, the Head of the Seed Testing Division in Northern Ireland, describes his aim as follows: "This book essays the rather delicate task of giving the non-technical an outline idea of the beautiful mechanism of reproduction in plants, setting before the agricultural student a syllabus of essentials to his seed studies, offering a skeleton guide to the professional business of seed testing, and indicating to the farmer or gardener the means available to him for cheap insurance of braird." The book opens with a simple account of the nature and development of

seeds (24 pages), and describes the various aspects of commercial seed production (33 pages), and the aims, technique and interpretation of the results of seed testing (40 pages). Crop and weed seeds are then described (47 pages) and, in a final chapter, (23 pages) Mr A. W. Munro of the Ministry of Agriculture and Fisheries gives a brief but useful account of The Seeds Act, 1920; its provisions and administration. There are two appendices containing, respectively, physical data of crop seeds, and notes and data for practical seed testing. The book closes with an index. There are four pages of text-figures and 15 plates, ten of the latter containing accurate and rather beautiful pencil drawings by the author of numerous crop and weed seeds as seen under a $\times 12$ pocket lens. The plates are not numbered but their contents are numbered in a tiresome manner. The author writes in an attractive style, his description of seed testing apparatus and procedure are brief but adequate, and his verbal portraits of seeds cogent and apt. He is a little irregular in his use of small and capital letters for specific names.

WILLIAM B. BRIERLEY.

General Plant Physiology. By E. C. BARTON WRIGHT. Pp. 539, 44 figures. London: Williams and Norgate, Ltd. 15*s.*

It is indicative of the new outlook on plant physiology as the dynamic branch of botany that after many years in which the only standard text-book on the subject was Palladin, revised and re-revised by Livingston, three text-books of major importance should have appeared in as many years. The tremendous strides which physiology has made in the last ten years, largely as a result of the new viewpoint which makes the study of the living plant as a co-ordinated entity the principal object, have emphasized the need for books presenting the subject from this modern angle.

Unfortunately, it is in just this respect that the work under review "misses the bus." The lay-out, the order of presentation and the whole conception of the book are admirable, but it fails in balance, critical exposition and modernity. It bears the marks of a work, the greater part of which was written some ten years earlier but left unpublished, and then issued with addenda. An analysis of the citations shows that out of nearly 700 references less than 18% are to papers dated 1930 or later, and many of these are referred to in the text only by brief paragraphs at the end of a section or chapter. As a result, recent work and views receive much less than their full share of attention and much is wholly omitted. For example, the work of Bolas and Goodall on the relation of assimilation to environmental factors, and that of Gregory and of Purvis on the physiological aspects of vernalization are not even mentioned, and these are only two of many cases of omission of really fundamental research of recent years.

As to the charge of lack of balance a single example will suffice. Nearly seven pages are devoted to an account of the controversy on mycorrhiza, the arguments and counter-arguments being set out in considerable detail, while the subject of dormancy in seeds, which is far more important physiologically, is dealt with inadequately in four pages, and dormancy of buds receives no mention at all despite the mass of literature which has been published on the subject.

As a text-book for students, however, perhaps the most serious criticism to be levelled at the book is that it is not a critical digest. Facts and theories are presented with no indications in many instances of the author's personal views. To be of use to first or second year students a work of the kind must be more than a text-book, it must be a guide-book. Advanced students may have developed a critical faculty, but how is an undergraduate to judge between the rival theories on, say, protein synthesis or the significance of transpiration? The student, faced with a brief statement of the experimental data and the conclusions drawn by different workers, may well feel himself lost in a morass.

A final criticism concerns the inclusion of statements which can only be attributed to carelessness in proof-reading. Thus on p. 133 we meet the surprising statement that

"in mesophytes, they (the stomata) are almost entirely confined to the lower surface of the leaf." Again, on p. 351, we find "in the leaf, the stomata open in darkness to get rid of excess of carbon dioxide", and on p. 449 the vernalization temperature for winter wheat is given as $-2°$ C. and in the next paragraph we read "Thus winter cereals must not be treated at a higher temperature than 2° to 3° C., and not below 0° C.", and later, on the same page, "An important practical point is that the seed *should not* be immediately sown after treatment" (italics ours) when in fact redrying before sowing is a disadvantage. Ordinary misprints are unfortunately also numerous, especially in the spelling of Latin names.

Parts I and II, which deal with the individual functions, the "classical physiology", of plants, are much better than Part III on growth, reproduction and irritability. The exposition of theories of respiration, and the chapters on osmotic pressure and permeability are particularly well done. As an historical approach to the subject, especially for advanced students, the work has real value and it will undoubtedly find a large public.

R. H. STOUGHTON.

Fundamentals of Biochemistry. By C. L. A. SCHMIDT and F. W. ALLEN. Pp. 388. London: McGraw-Hill Publishing Co., Ltd. 1938. 18*s*.

Of students' text-books of practical biochemistry the two in the English language which are best known are almost certainly "Cole"—a favourite in Great Britain—and "Hawk" which occupies a very similar position in the United States. Both have undergone familiar evolution in the course of the nine (Cole), or eleven (Hawk) editions which have so far appeared. They have started as rather thin, almost entirely "practical" volumes of laboratory experiments, and from edition to edition have steadily increased in size as the amount of text required as background and explanation of the strictly experimental sections has of necessity increased.

The present students' text-book by Schmidt & Allen of the University of California belongs to the general category of the evolved "Cole" or "Hawk". More than that, it belongs to the same high class, which is no mean commendation, and, like the modern editions of these books, combines a clear and straightforward text with a well-digested course of laboratory experiments. Since it is a first edition it has an added freshness of outlook, and is able to concentrate on those sections of biochemistry which are at present the growing points.

From the student's standpoint the general arrangement of the book is most useful. Key papers with which the student should become familiar, in the research literature in each field, are referred to early in most of the chapters. Then a synopsis follows of the essentials of the particular aspect of biochemistry with which the chapter deals, and finally a number, not too many, of experiments on modern lines are described, to be carried out by the individual student or by a small group of students. Of the experiments suggested, several of which are new to any student text-book, some gain force and training value from the fact that they may be carried out, without danger, by the student on himself.

The subjects of chapters include neutrality regulation in the body, enzymes, mineral metabolism, vitamins, endocrines, lipids, bile, carbohydrates, amino acids and proteins, urine and blood analysis, and energy exchange. A few illustrative numerical problems are stated and solved in an appendix. Here it might have been better to have removed the solutions (which are now given in close juxtaposition to the problems) to another part of the appendix. Apart from this minor criticism the reviewer has nothing but praise for a sound, well-arranged text-book of practical biochemistry, a book with flavour and character, and one particularly suited to giving the junior and intermediate student of biochemistry a sound training in laboratory methods.

H. D. KAY.

The Observer's Book of Trees and Shrubs of the British Isles. By W. J. Stokoe. Pp. 240, with 177 illustrations, 16 in colour. London and New York: Frederick Warne and Co., Ltd., 1938. 2*s*. 6*d*.

Descriptions are given of all the British trees, and of several introduced trees commonly grown in British woods and gardens: 106 species are described. The descriptions are accurate and are supplemented by illustrations of the whole tree, the trunk, and in most cases, of the leaves, flowers and fruit. The botanical name and family of each species are given and, with the exception of the misspelling on three different pages of "*araucana*", there are very few misprints. The book is of a convenient size for the pocket and the excellent descriptions and illustrations should enable any interested observer to identify correctly the trees usually grown in this country.

A. G. ERITH.

Mother Earth. By G. W. Robinson. Pp. viii + 202, with 6 figures, 2 maps and 1 plate. London: Thomas Murby and Co. 1937. 5*s*. 6*d*.

Prof. Robinson of Bangor is well known as an authority on "soil science" or, as he regards this term as a barbarism, on pedology. His previous volume, *Soils, their Origin, Constitution, and Classification,* of which a second edition was published in 1937, is a standard treatise more suitable for pedologists and agricultural chemists than for workers in other fields or general readers but, as Prof. Robinson believes that "the general reader, perhaps even the farmer and the landowner" ought to be interested in the soil, he has written the present book for their instruction and delectation. In order to express his ideas in a more intimate and direct way than by formal writing he has cast his thoughts into the form of "Letters on Soil" addressed to Prof. Stapledon. He says: "The letters I am about to write will form no systematic course of instruction. They will not form even an elementary text-book of pedology. They are to be considered rather as a series of essays, or even meditations, on topics relating to the soil." "I believe that the story of the soil is as interesting as that of any other part of the great Universe, even though its transactions are fulfilled beneath our feet." And so, in seventeen letters, he discourses on the natural philosophy of the soil, and has produced a book that will do more than all the text-books to interest people in the soil for its own sake, and lead them to think rightly on matters which touch their daily life so intimately.

Commencing with soil materials and structure the author passes to the soil profile and some typical soils, various physical factors, and manures and fertilizers, and shows how all these relate to soil fertility and crop growth. A discussion of soil surveys leads to a consideration of the natural vegetation of the country, agricultural soils, waste lands, and soil destruction and conservation. In a concluding letter the author presents some more personal reflexions on the relation of science to agriculture and to rural policy.

This synopsis gives no idea of the wide scope and thoughtful quality of the book or of the matured knowledge and wisdom it contains. It is finely written and it is a delight to read. At the same time it presents an interesting and beautifully clear picture of achievements, aims and viewpoints in pedology.

WILLIAM B. BRIERLEY.

Genetics and the Origin of Species. By T. Dobzhansky. Pp. xvi + 364. New York: Columbia University Press. (London: Oxford University Press.) 1937. 18*s*.

Genetics has developed so rapidly and centrifugally and, in many of its aspects, has become so abstruse, that even the geneticist finds difficulty in breasting the flood. And yet, genetics is so significant for all immediate problems of biology, and has such

far-reaching implications in biological theory, that no biologist dare neglect its advance. For immediate purposes one can pick out occasional genetic researches and attempt to correlate their data with one's own. But there is no intellectual satisfaction in this course, and its superficiality and empiricism may lead to grievous error. One needs to see genetics in its biological setting, in perspective against the general background of the study of living things: one needs to appreciate its relationships and trends, its impacts on cognate or apparently diverse issues, its significance for immediately practical or more remote problems of biology, and its implications in biological theory. The practical and immediate application of genetic knowledge and viewpoints in all aspects of plant culture is obvious, but equally is it obvious that genetic theory and data have direct bearing on biological philosophy, on, for example, the theory of evolution. Yet, genetics is an experimental study of the laboratory and breeding pen, with immediate results and empirical viewpoints, whilst evolution is an observational study of the museum and field, with long scale results and philosophic viewpoints. What is the relation between cytogenetics and natural history; between for example, the immediate micro-evolutionary phenomena of laboratory cultures of *Drosophila* and the macro-evolutionary phenomena of elephant phylogeny in geological time? Can the laboratory methods and phenomena be put to field test or correlated with phenomena in Nature? For any discussion of such problems most of us are dependent upon the more general writings of natural historians or geneticists, and we can only hope that our guides are reliable. Prof. Dobzhansky seems to be equally at home in both fields and a reading of his book gives one the impression that his guidance is trustworthy.

He circumscribes his task as follows: "The present book is devoted to a discussion of the mechanisms of species formation in terms of the facts and theories of genetics... and to see how much of evolution in general can be adequately understood on this basis."

In a first chapter on "Organic Diversity" the author clears the ground and defines three levels of the evolution process: evolutionary statics, or the forces bringing about changes in the genetic composition of populations; evolutionary dynamics, or the interactions of these forces in race and species formation and disintegration; and, thirdly, the realm of fixation of the diversity already attained on the preceding two levels. Evolutionary statics is dealt with in Chs. II, III and IV, devoted, respectively, to gene mutation, mutation as a basis for racial and specific differences, and chromosomal changes. These pages contain, in short compass and readable language, the most critical appraisal of the wider evolutionary relationships of these problems I know.

From this survey the author concludes that gene mutations and numerical chromosome changes are the principal sources of variation. Hereditary variation, thus produced and thrust into a population, enters into the field of action of factors on a different level from those producing variation. These are selection, manner of breeding, and environmental relationships, factors belonging to the realm of the physiology of populations, and concerned with evolutionary dynamics. In Chs. V and VI, therefore, the different agents influencing the fate of the genetic diversity in natural populations are analysed, Ch. VI on "Selection" in particular being a beautifully clear and balanced exposition of this problem.

The next three chapters concern, in general, the third evolutionary level, the fixation of diversity. Ch. VII is an excellent discussion of polyploidy, or the cataclysmic origin of species, the relative prevalence of which in plants and scarcity in animals constitutes the greatest known difference between the evolutionary patterns in the two kingdoms. Ch. VIII is a clear and convincing discussion of the geographical and physiological isolating mechanisms which prevent the interbreeding of groups of individuals, while Ch. IX considers hybrid sterility.

Ch. X on "Species as Natural Units" will, to many readers, be the most interesting but least convincing in the book, and one could wish that the author had allowed himself more scope for the elaboration of his views. Throughout the work he emphasizes the dynamic nature of the species concept, "Species is a stage in a process, not a static unit," and he now defines the species as "That stage of evolutionary

process at which the once actually or potentially interbreeding array of forms becomes segregated in two or more separate arrays which are physiologically incapable of breeding." Such a definition, obviously, is more suitable for animals than for plants and, further, it raises immediately the question of asexual and apogamic organisms. Of these the author says "As in cross-fertilizing organisms, the biotypes in the asexual ones are clustered...the clusters are arranged in a hierarchical order...the different clusters may, then, be designated some as species, others as sub-genera, still others as genera, etc. Which one of these ranks is ascribed to a given cluster is, however, decided by considerations of convenience, and the decision is in this sense purely arbitrary. In other words, the species as a category which is more fixed, and therefore less arbitrary than the rest, is lacking in asexual and obligatorily self-fertilizing organisms. All the criteria of species distinction utterly break down in such forms." Some botanists may find this conclusion rather startling.

Prof. Dobzhansky has written a most thoughtful and stimulating book, and he has a capacity of making difficult things clear without falsifying them by over-simplification, and of putting his ideas concisely and interestingly in simple language. For geneticists the book will be valuable as a clarification of ideas and viewpoints whilst, for those of us who are just ordinary botanists or zoologists, it will be invaluable as enabling us to visualize our science in a wider and deeper perspective. The book contains a useful bibliography which, however, shows surprising omissions.

WILLIAM B. BRIERLEY.